

Journal of Applied Microscopy and Laboratory Methods

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No. 6

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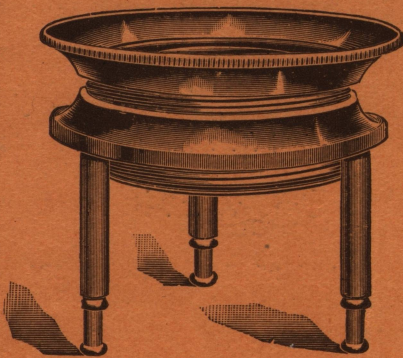
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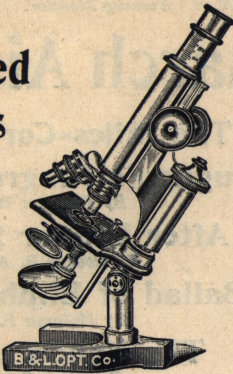
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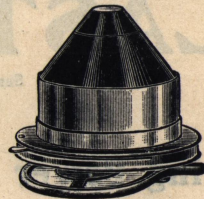
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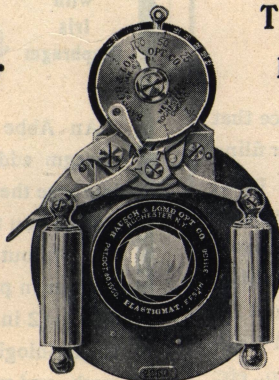
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Improved Automatic Microtomes.

The two microtomes, to which I wish to call attention, are modifications of the two forms of automatic microtomes described by me in 1897.¹ In neither instrument have the essential features of the construction been changed, but the alterations made were introduced partly to increase the accuracy of the cutting, partly to facilitate the manipulation of the apparatus. Messrs. Bausch & Lomb undertook the series of improvements at my request, and I am indebted especially to Mr. Edward Bausch for the time and thought he has given both to planning and executing the work involved. Every detail has been the subject of extended consultation, but I wish the pleasure of acknowledging that several valuable innovations were first suggested by Mr. Bausch. The new feed for the precision microtome was devised and worked out by Messrs. Bausch & Lomb.

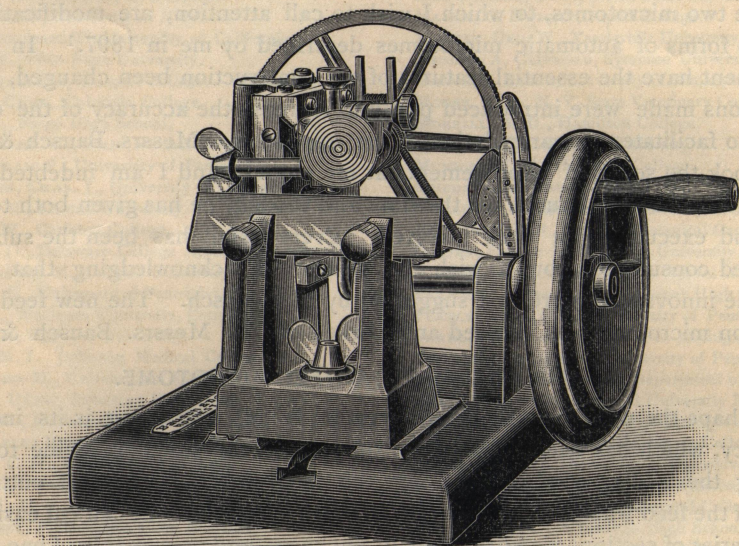
I. THE AUTOMATIC WHEEL-MICROTOME.

Perhaps the most important improvement in this instrument is its increased accuracy, which has been secured by the use of the finest machine tools for planing the sliding surfaces, cutting the micrometer screw and cutting the teeth of the feed wheel. The accuracy is now so great that one can cut easily a uniform series of sections of two microns in thickness, and presumably of one micron, but the one micron sections I have not sufficiently tested. In the former instruments, both American and German, the sections would skip occasionally, and then the following section would be of nearly double thickness and the uniformity of a series ruined. With the present instruments, three of which I have tested, this vexatious irregularity does not occur, at least with ordinary objects. I have not yet tried the microtome with objects specially difficult to cut.

Other improvements have rendered the microtome more convenient to use. The following five changes are most important: *First*—The toothed wheel which supplies the automatic feed has been enlarged and cut to have five-hundred teeth, so that, as the micrometer screw has a half-millimeter pitch, each tooth equals a feed of one micron. *Second*—The automatic feed has been so contrived that it will give any desired thickness, from one to twenty-five microns, and can be changed in a moment. This is accomplished by having the pawl-

¹ SCIENCE, Vol. V, No. 127, pages 857-866, June 4, 1897.

bearing lever strike against an eccentric cam, which has twenty-five stops in it. To diminish the wear and friction, the lever where it strikes the cam is furnished with a steel wheel. To prevent the dislocation of the pawl it is made in the form of a fork, the prongs of which fit over the edges of the toothed wheel, so that the pawl cannot slip to either side. *Third*—To prevent the overthrow of the wheel, which, in rapid section cutting, is apt to cause a greater feed and therefore sections of greater thickness than intended, a simple and effective brake has been added, the tension of which is easily regulated. The brake consists of two steel springs, each with a leather pad, which press against the rim of the wheel by a set-screw; these pads may be pressed together more or less, thus regulating their pressure upon the wheel between them. *Fourth*—A split nut has been provided for the micrometer screw, the two pieces of the nut are attached to levers, which work like pincers, so that by pressing the levers the nut is opened and object carrier may be run forward or back rapidly without



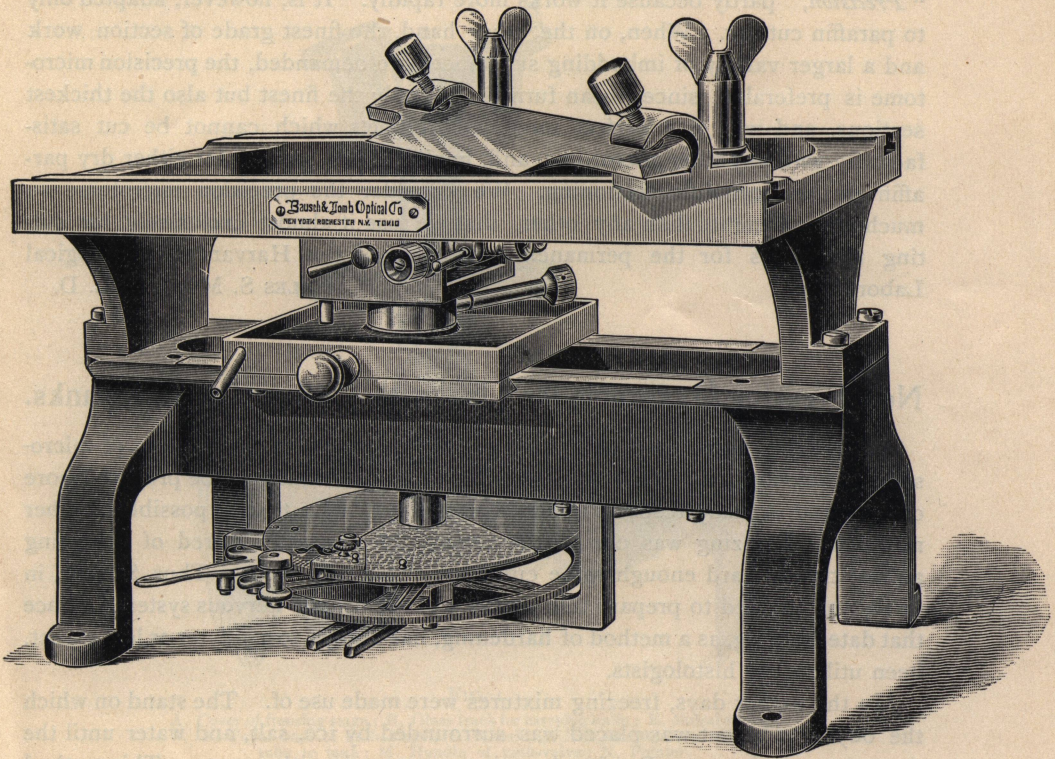
disturbing the screw; by releasing the levers the nut closes, and as it closes snaps into place automatically. The device is such that when the carriage is run way back to the beginning of its excursion, the nut snaps into place of itself, and the machine is ready to work. *Fifth*—The main wheel by which the machine is worked, has been so carefully balanced that the microtome may be stopped at any point, and will remain in the same position without change.

There are other minor alterations, which do not call for special description. The resulting apparatus produced by Messrs. Bausch & Lomb is a fine instrument of precision, very convenient and satisfactory in use, and attractive in appearance.

II. THE PRECISION MICROTOME.

The original form of this instrument was found by long continued use to have certain minor defects, three of which caused inconvenience. Perhaps the

most serious of them was the liability of the automatic feed to get out of order unless great and constant care was taken in the use of the machine. The second defect was the wear on the ways, which took place chiefly in the middle, and very little or not at all at the ends, so that the carriage was liable to bind at the end of a longer excursion than usual. A third defect was that the object holder could be lowered only by the slow process of turning the micrometer screw backwards. Messrs. Bausch & Lomb have sought to remedy the first defect by a new feeding device, which cannot be clearly described without special illustrations. The general principle is to have a lever bearing a pawl, which moves the toothed wheel; the backward motion is so arranged that the pawl is lifted free from the wheel altogether, but at the end of the backward motion the



pawl is brought into place against the wheel again, by an action of the lever. For this purpose the lever is hinged in its middle, so that its outer arm can bend independently in one direction without displacing the whole lever. The motion of the outer arm is utilized to bring the pawl into place against the toothed wheel. This wheel is provided with five hundred teeth, each tooth equaling a feed of one micron. It is also, to prevent overthrowing, supplied with a brake similar to that on the wheel microtome. Of the new automatic feed it is proposed to publish a separate account, with figures, on another occasion. The second defect, that of the ways, has been obviated very simply by shortening the ways themselves so that the whole of the ways will be worn

during each ordinary excursion of the carriage. The third defect has been met by the addition of the split-nut; it is only necessary to press upon the levers which open the nut, in order to allow the object holder to sink gently to a lower level. Other minor improvements have been made, of which I will mention only the spring-buffers, which prevent the carriage, if it be moved a little too far or fast, from hitting too violently against the frame of the microtome. The other improvements have been intended chiefly to increase the rigidity of the apparatus.

The two instruments, above described, seem to me better suited to meet the severer requirements of microtomic work, than any others which I have hitherto tested. The "*Wheel*" microtome will probably be more used than the "*Precision*," partly because it works more rapidly. It is, however, adapted only to paraffin cutting. When, on the other hand, the finest grade of section work and a larger variety of imbedding substances are demanded, the precision microtome is preferable, since it can furnish not only the finest but also the thickest sections, and will give perfect sections of objects which cannot be cut satisfactorily with any other microtome, and, finally, it can be used for either dry paraffin or wet celloidin sectioning. We consider the precision microtome so much more accurate than any other, that we use it almost exclusively for cutting the series for the permanent collection of the Harvard Embryological Laboratory.

CHARLES S. MINOT, LL. D.

New Freezing Microtome for Use with Carbon-Dioxide Tanks.

A freezing microtome offers two great advantages to the student of microscopic anatomy. By its use thin sections of animal tissues can be prepared more quickly and in many respects in a less altered condition than is possible by other methods. Freezing was one of the earliest methods discovered of rendering animal tissues hard enough to be cut readily into thin slices. Thus, Stilling, in 1843, was enabled to prepare thin sections of the central nervous system. Since that date freezing, as a method of hardening, has always, to a greater or less extent, been utilized by histologists.

In the earlier days, freezing mixtures were made use of. The stand on which the object to be cut was placed was surrounded by ice, salt, and water until the tissue became frozen. Freehand sections were made with a razor. This method of freezing tissues for microscopic work was superseded by methods which involve the use of volatile fluids like ether. Instruments for the utilization of these fluids were devised as attachments to the precision microtomes which were invented after the use of celloidin and paraffin as embedding agents was discovered. These instruments are still in use among histologists. In the hands of careful workers they give satisfaction. They are, however, slow in action, expensive to use, and easily put out of order. For these reasons, although almost all biologists have freezing attachments to their sliding microtomes, few make much use of them. Of late, carbon-dioxide has been much utilized, especially by pathologists, as a means of freezing tissues for sectioning. The convenience with which

fluid carbon-dioxide may be obtained in tanks, and its power of rapid freezing, have caused it to be preferred to ether and similar fluids. In every active pathological laboratory the freezing microtome is in daily use. Perhaps its greatest value lies in the fact that thin sections may be made within a few minutes after the removal of tissue from the body, and in a few minutes more these sections may be hardened, stained, cleared, and mounted. The surgeon may thus be given a positive diagnosis of the microscopic condition of diseased tissues while he proceeds with an operation.

The carbon-dioxide microtomes commonly used have, however, several drawbacks which have served to render them far less useful than they should be. From the practical standpoint their most serious drawbacks are a tendency to

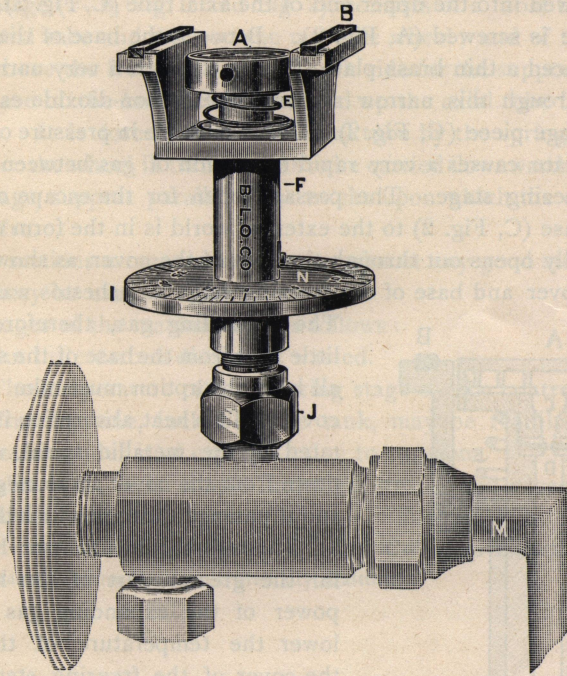


FIG. 1.

A. Cover of freezing stage ; B. Glass track for carrying knife ; E. Spiral spring ; F. Tubal base of knife-stage ; I. Wheel ; J. Nut for attaching axial tube to tank ; M. Handle of tank-valve ; N. Pointer.

become clogged and a great wastefulness of gas. From the scientific standpoint, lack of control over the temperature of the freezing stage serves to give rise to an "over-freezing," which produces marked alterations in the tissues. In order to remedy these defects the machine described below was devised. In designing a practical machine I had the able assistance of Mr. E. F. Northrup. The Bausch & Lomb Company, who have undertaken its manufacture, have also offered suggestions that have proved of much value.

Figure 1 shows the machine as it stands ready for use. It is supported directly by the nozzle of the carbon-dioxide tank. This offers a firm and convenient means of attachment, but, if desired, a heavy tubing may be utilized to

connect tank and machine. When the microtome is screwed directly upon the carbon-dioxide tank it is necessary that the latter lie in a horizontal position. On the other hand, if an L-shaped piece of tubing be utilized to connect freezing microtome and tank, the tank may be placed at any desired angle.

The valve of the tank is used to control the escape of gas into the machine.

The axis and main support of the instrument consists of a stout tube with a narrow lumen (K-D, Fig. 2). This axial tube is united by a nut (J, Figs. 1 and 2), either directly to the nozzle of the tank, or, in case a connecting tube is used, to the latter.

On the top of the axial tube the freezing stage (A, Fig. 1, A-C, Fig. 2), is screwed. This stage piece consists of two parts, a base and a cover. The base is the part screwed into the upper end of the axial tube (C, Fig. 2). To this base the cover piece is screwed (A, Fig. 2). Between the base of the stage and the axial tube is placed a thin brass plate (D, Fig. 2), with a very narrow aperture at its center. Through this narrow aperture the carbon-dioxide escapes into the lumen of the stage piece (C, Fig. 2). The difference in pressure on the two sides of the brass plate causes a very rapid expansion of gas between the cover and base of the freezing stage. The passage open for the escape of gas from the lumen of the base (C, Fig. 2) to the external world is in the form of a spiral passage which finally opens out through the side of the cover, as shown in Fig. 1, A. Between the cover and base of the freezing stage an asbestos washer is placed.

The expanding gas, therefore, can absorb little heat from the base of the stage. Almost all heat absorption must take place from the cover. This heat absorption is greatly facilitated by the metallic spiral, which projects down from the cover so as to give rise to the spiral passage through which the gas escapes.

Through the mechanism here described far the greater part of the heat absorbing power of the expanding gas is utilized to lower the temperature of the surface of the cover of the freezing stage. The temperature of the rest of the machine is but little altered. Good control of the temperature of the freezing stage can be thus maintained. This control is further rendered possible by the valve of the tank. If this valve be turned on full the temperature of the cover of the freezing stage will be quickly reduced to a very low point. Tissue placed on it is quickly frozen. On the other hand, if the gas is not permitted to escape from the tank with full force, the difference in pressure on the two sides of the brass plate is less and heat absorption from the cover is less marked.

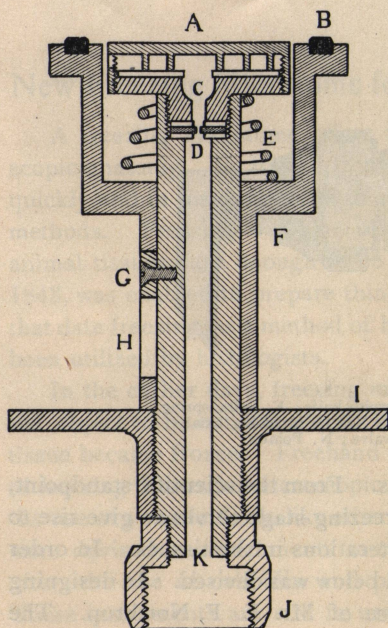


FIG. 2.

A. Cover of freezing stage; B. Glass track for carrying knife; C. Aperture in base of freezing stage; D. Aperture in thin brass plate; E. Spiral spring; F. Tubal base of knife stage; G. Check for limiting movements of knife-stage; H. Groove for G; I. Wheel; J. Nut for attaching axial tube to tank; K. Opening into lumen of axial tube.

In this way tissues placed on the cover may be slowly frozen without subjecting them to severe cold. Thus, too, a constant low temperature may be maintained by opening the tank valve to the required point.

The mechanism for controlling the thickness of the sections is equally simple. On the lower end of the axial tube a movable wheel (I, Fig. 1 and Fig. 2) is placed. This wheel moves up and down the axial tube on a screw thread, cut twenty-five threads to the inch. A complete revolution of the wheel, therefore, raises or lowers it a millimeter. The margin of the wheel is divided into fifty spaces, each of which therefore represents twenty microns. A pointer (N, Fig. 1) serves to indicate the number of spaces passed in a partial revolution of the wheel, and thus to show the thickness of the sections cut.

The knife-stage (F-B, Fig. 1 and Fig. 2), consists of a tubal base (F), which surrounds an axial tube and rests on the movable wheel; and of two flanges (B), which extend above the freezing stage on each side for the support of the cutting blade. The base of the knife stage is moved up the axial tube by screwing the wheel upwards. It is forced down the axial tube by the spring (E, Fig. 1 and Fig. 2) whenever the wheel is turned so as to be carried downwards. The flanges of the knife-stage support parallel glass tracks upon which the cutting blade is carried to and fro.

For cutting sections a razor or a plane, or almost any good steel blade with a straight edge, may be used.

The advantages of the machine are as follows:

1. But little carbon-dioxide is wasted.
2. The temperature of the freezing stage can be controlled.
3. The machine, including the tank, may be readily carried about.

This should render it of especial value to surgeons.

4. Above all, it is simple in design, strong, and exceedingly unlikely to get out of order.*

CHARLES RUSSELL BARDEEN.

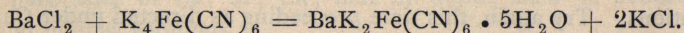
Anatomical Laboratory, Johns Hopkins University, Baltimore.

MICRO-CHEMICAL ANALYSIS.

XIV.

BARIUM—Continued.

III. Barium unites with Potassium Ferrocyanide to form a Ferrocyanide of Barium and Potassium.

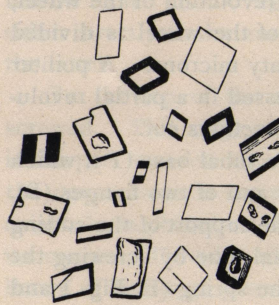


Method.—To the test drop, which should contain no free mineral acid, add a little acetic acid, then a little potassium ferrocyanide, and warm the preparation very gently. In a few seconds rhombs of the double ferrocyanide will appear

* The description of the microtome here given is essentially similar to one that will appear in the May-June number of the Johns Hopkins Bulletin, 1901.

near the edge of the test drop (Fig. 57). These crystals are clear and transparent. By transmitted light they appear to be colorless, but if examined by reflected light they will be seen to have a very faint, almost imperceptible yellow tint.

Remarks.—The reagent crystallizes in prisms belonging to the monoclinic system, while the barium salt is to be ascribed to the orthorhombic system. The danger of confusing the two salts is, therefore, slight. The crystals of the ferrocyanide of barium and potassium extinguish parallel to the diagonal bisecting the acute angles of the rhombs. Many of the crystals of the barium salt appear to be rectangular plates or even cubes, according to the position in which they are seen. An examination with crossed nicols will dispel the illusion. When the test drop is concentrated with respect to barium, the crystals of the double ferrocyanide separate at the point where the reagent was introduced.



1 Div. = 0.01 mm.

Fig. 57.

Potassium ferrocyanide, though giving a neat reaction with pure salts of barium, is of little value when dealing with mixtures. It is then often difficult to avoid the precipitation of calcium with the barium, particularly if much ammonium chloride is present, or if much sodium acetate has been added to mitigate the action of mineral acids.

From mixtures, strontium may sometimes be precipitated if the solution is quite concentrated, and may thus interfere with the test. Pure salts of strontium give, in very concentrated solutions, only a granular deposit consisting of globular masses, exhibiting no distinguishable crystal form.

Magnesium is precipitated from ammoniacal solutions, but neither from acid nor from neutral solutions; hence the presence of this element will not mask the test for barium.

In addition to calcium and strontium, there are a number of other elements, which, if present, will either be precipitated in insoluble form or will interfere with the formation of the barium crystals. In this list the most frequently met with will be lead, iron, zinc, rare earths, and less often copper, mercury, uranium, titanium.

Exercises for Practice.

Crystallize a little of the reagent alone, and determine its optical properties.

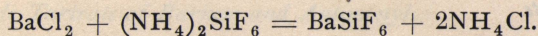
Try reagent on pure salts of Ca; Sr; Ba; using both dilute and concentrated solutions. Try again, this time proceeding as directed under Calcium.

Try the reagent on mixtures, say of Ca and Sr; Ca and Ba; Sr and Ba.

Try effect of the reagent on salts of Pb, Zn and Fe. Then make mixtures of Ba and these elements, and test.

Make a preparation of $\text{BaK}_2\text{Fe}(\text{CN})_6 \cdot 5\text{H}_2\text{O}$, measure the angles of the crystals, and determine the optical properties of the compound.

IV. *Ammonium Fluosilicate precipitates Barium Fluosilicate.*



Method.—Place, on a celluloid slip, a drop of the moderately dilute solution to be tested. Acidify with acetic acid; spread out the drop a trifle; add a fragment of ammonium fluosilicate and warm gently. There will immediately form, throughout the test drop, fusiform crystals, either singly, in crosses, or in more or less irregular masses (Fig. 58).

If the solution is quite dilute, instead of the usual fusiform crystals, well-defined rhombohedra and prismatic crystals are obtained.

Remarks.—See Sodium, Method III. It is important to avoid testing concentrated solutions, since fluosilicates of calcium or strontium may possibly separate, although neither of these elements will be precipitated under the conditions which usually obtain in testing. This caution as to concentration is necessary, because when crystals of calcium fluosilicate $\text{CaSiF}_6 \cdot 2\text{H}_2\text{O}$ do appear, the forms obtained may resemble the barium salt quite closely. Calcium fluosilicate is to be referred, however, to the monoclinic system. The corresponding strontium salt, $\text{SrSiF}_6 \cdot 2\text{H}_2\text{O}$, is isomorphous with the calcium compound, and is slightly less soluble than the latter.

The form of barium fluosilicate varies quite a little according to the concentration of the test drop, and to its state of acidity.

Much free mineral acid is apt to interfere slightly with the precipitation.

When employing celluloid slips, it is of course essential to use great care in warming the preparation, owing to the inflammability of the material. Under proper precautions there is very little danger of losing the test. The warming should be slight.

If barium alone is to be searched for, a glass slip may be employed, as the formation of any sodium fluosilicate will not materially affect the test for barium.

In the absence of ammonium fluosilicate, ammonium fluoride and a little silica may be added to the test drop, or the silica may be suppressed and the test performed on a glass slip.

Exercises for Practice.

Test pure salts of Ba; Sr; Ca; first in dilute, then in concentrated, in neutral, and in acid solutions.

Make a mixture of Ca, Sr, Ba, and test as above.

Try reaction on mixtures of Na and Ba; then on Na, Ca, Ba; Na, Sr, Ba; varying the concentration of the test drops.

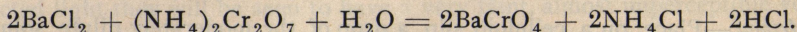
Test a mixture of Ba and Mg; then one of Ba and Fe.

Try the reagent on a salt of Pb.



Fig. 58.

V. Ammonium Dichromate added to solutions containing Barium precipitates Barium Chromate.



Method.—Employ a very dilute solution of the barium salt. Add a little acetic acid, and then a small fragment of the reagent. Do not stir. In adding the reagent avoid scratching the glass slide with the glass rod or platinum wire.

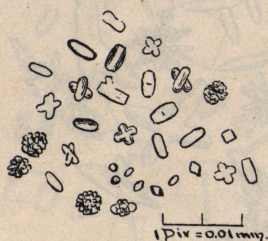


Fig. 59.

Barium chromate separates in the form of very minute, light yellow, globular masses, and tiny rods with rounded ends. These rods are often arranged in crosses and Xs (Fig. 59).

Occasionally rectangular plates are obtained.

Remarks.—The complete precipitation of all the barium present is slow.

Strontium will not separate in acid solutions, and calcium not even in the presence of ammonium hydroxide.

When strontium is to be tested for as well as barium, the test drop, after the addition of the reagent and examination for barium, is gently heated, then allowed to stand for some time. The supernatant solution is drawn off, and to it a tiny fragment of the reagent is again added, and the preparation warmed; if no precipitate results, showing that all the barium has been removed, strontium can be tested for by adding ammonium hydroxide.

In warming the preparation to accelerate the separation of the barium salt, great care must be exercised in order to avoid concentrating the drop to a point where strontium might be precipitated.

It is often better to allow a drop of ammonium hydroxide to flow slowly into the side of the test drop, rather than add it at once to the center of the preparation.

Normal potassium chromate produces, with barium salts, a precipitate similar to that obtained with dichromate, but is not to be recommended as a reagent because of its property of also precipitating strontium compounds in acid solution.

Ordinarily the precipitate of barium chromate is largely amorphous in appearance. Here and there, however, will be found spots where there are recognizable crystals. A high power is always required for the recognition of the form of the crystals, hence the drop to be studied must be spread out quite thin.

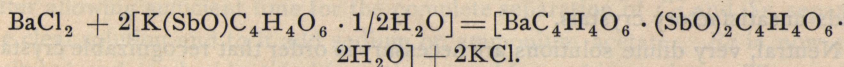
Free mineral acids interfere with the test.

Exercises for Practice.

Try reaction on salts of Ba; Sr; Ca; in acid, neutral and ammoniacal solutions, both in concentrated and in dilute solutions.

Try mixtures of Ca and Ba; Sr and Ba; use solutions acidified with acetic acid, draw off the clear solution, and to it add ammonium hydroxide.

VI. *Potassium Antimonyl Tartrate precipitates, from solutions containing Barium, a Double Tartrate of Barium and Antimonyl.*



Method.—To the neutral and moderately concentrated drop to be tested add a small drop of acetic acid. Place close to the test drop a drop of distilled water containing the reagent. Warm the reagent drop, and stir until all the tartar emetic has dissolved. Warm the test drop, and while both drops are warm cause the reagent to flow into the solution being examined. On cooling, crystals of the double tartrate will separate in masses near the edges of the drop. As soon as crystals appear draw a platinum wire through one of the crystal masses, and thence across the drop. This will induce crystallization along the path of the wire and will lead to the formation of well formed single crystals. When this procedure is followed, beautiful, clear cut, thin, transparent, colorless crystals of the orthorhombic system are obtained. The usual forms are rhombs and hexagons (Fig. 60); the latter result from the cutting off of the acute angles of the rhombs. Multiple twins are frequent. There is a great tendency toward the formation of aggregates.

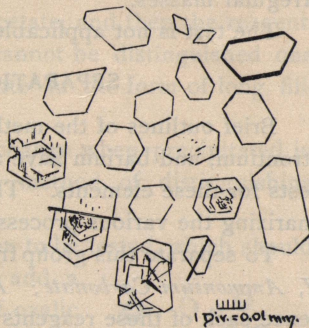


Fig. 60.

Remarks.—Free mineral acids must be absent.

It is unfortunate that this neat reaction is of quite limited application.

Strontium may be precipitated in like forms if the conditions are favorable.

Calcium interferes, as do also members of Group I, and of the magnesium group.

When dealing with small amounts of barium in a mixture, it is necessary, owing to the solubility of the barium double salt, to concentrate the solution to such a point as to render it practically impossible to obtain satisfactory results, because of the crystallization of other compounds.

Lead forms a double antimonyl tartrate isomorphous with that of barium and strontium. *

Of the other heavy metals, silver is the only one which will yield a crystalline precipitate with the reagent, but under the conditions of the test as described, the precipitate with silver is usually amorphous.

Exercises for Practice.

Dissolve a little of the reagent, and allow it to crystallize. Study the crystal forms, and determine their optical properties.

Try the reaction on BaCl_2 ; CaCl_2 ; SrCl_2 .

Test mixtures of Na and Ba; Mg and Ba; Ca and Ba.

Try action of the reagent on salts of Pb.

* Traube, Zeit. Kryst. 26: 188.

VII. With Primary Sodium Carbonate or Ammonium Carbonate.

The latter reagent gives much better results, but even at its best the reaction yields unsatisfactory crystals.

Neutral, very dilute solutions are necessary in order that recognizable crystals shall be obtained. The sodium salt tends to produce minute, spider-like aggregates and spherulites.

Ammonium carbonate yields tiny spindle-shaped crystallites, dumb-bells, and irregular masses.

The test is not applicable in the presence of Ca, Sr, Mg, Li, etc.

SEPARATION OF THE CALCIUM GROUP.

Brief outlines of the methods for the separation and identification of calcium, strontium, and barium have already been given in the discussion of the various tests for these elements. There remains, therefore, only the necessity of summarizing the various processes.

To separate this group from other elements, three reagents can be employed: *I, Ammonium Carbonate; II, Oxalic Acid; III, Sulphuric Acid.* For convenience each of these reagents will be discussed separately and in turn.

I. Ammonium Carbonate in Ammoniacal Solution.

In addition to *Ca*, *Sr*, and *Ba*; there can also be precipitated a number of other elements and compounds. Chief among these should be mentioned, *rare earths, Mn, Cr, Al, Fe, Pb, Magnesium group, phosphates, borates, arsenates, molybdates, oxalates, tartrates*, etc.

Inasmuch as the tests for the elements other than those of Group I and the Calcium group have not yet been described, it is not deemed expedient at this point to enter into a discussion of the methods for dealing with complicated mixtures.

The clear liquid is drawn off, or otherwise separated from the precipitate produced by the reagent. The precipitate is washed, and dissolved in hydrochloric acid.

Test one portion of the hydrochloric acid solution with sulphuric acid for *Ca*, if an amorphous or granular precipitate results, *Sr* or *Ba* (or *Pb*) is present, or the substance may contain both.

Test a second portion with ammonium fluosilicate for *Ba*.

If no *Ba* is found, test for *Sr* with ammonium dichromate and ammonium hydroxide.

If *Ba* is present, precipitate this element with dichromate in acid solution, Draw off and test for *Sr* with ammonium hydroxide.

II. Oxalic Acid.

Three modifications can be satisfactorily employed, the choice being governed by the nature of the material.

- a.* Precipitation with oxalic acid in nitric acid solution.
- b.* Precipitation with oxalic acid in the presence of ferric chloride.
- c.* Precipitation with oxalic acid in the presence of stannic chloride.

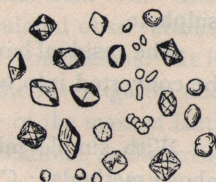
a. To the test drop add a little nitric acid, then the reagent. *Ba* is not precipitated. *Ca* and *Sr* separate slowly in the usual form of their oxalates. After allowing sufficient time for the complete separation of *Ca* and *Sr*, separate the clear solution and to it add sodium or ammonium acetate. *Ba* is now precipitated, and can be identified from the crystal form of its oxalate. The precipitated oxalates of *Ca* and *Sr* can be tested at once by adding sulphuric acid, or they can be dried, heated, and thus converted into carbonates. The carbonates can be dissolved in acid, and the solution thus obtained tested.

b. To the test drop add ferric chloride sodium acetate, and then the reagent. *Ca* and *Sr* appear in their normal form, and hence cannot be distinguished one from the other; but *Ba* separates as a double oxalate in the form of long filiform crystals of characteristic appearance.

c. Oxalates of *Ca*, *Sr*, *Ba* undergo a marvelous change when precipitated in the presence of stannic chloride. This beautiful method of distinguishing between these elements is due to Behrens.

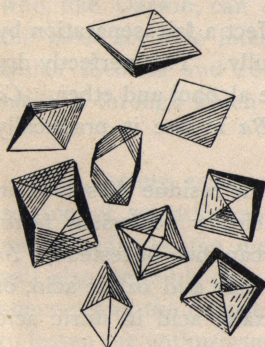
To a drop of the moderately concentrated solution to be tested, which should be neutral, or at the most only very faintly acid, add a little stannic chloride; stir, then add a fragment of oxalic acid.

Instead of the usual crystal forms, the oxalates separating in the presence of stannic chloride undergo a remarkable change. *Ca* yields rounded and oval grains and thin disks, with here and there crystals showing unmistakable evidence of trying to develop into octahedra. The crystals are never of large size, though larger than those of the normally formed oxalate, and apparently never grow into clear cut octahedra (Fig. 61). *Sr* under the same conditions yields large octahedra (Tetragonal), clear cut and beautifully developed (Fig. 62). These crystals



Div. = 0.01 mm.

Fig. 61.



Div. = 0.01 mm.

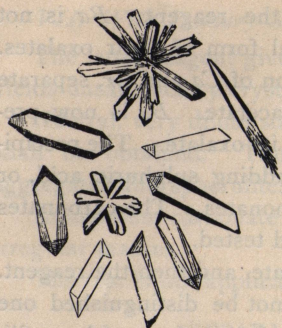
Fig. 62.

soon become corroded, and may eventually disappear; hence it is necessary to examine the preparation immediately after the addition of the oxalic acid. Too much free mineral acid, or an excessive amount of the stannic salt, interferes with the reaction.

Ba is precipitated by oxalic acid under the above conditions as neat, well developed prisms, singly, in crosses, and in radiating masses (Fig. 63). If much *Ba* is present, long, very pointed, fusiform crystals result, and bundles of slender, pointed needles.

Mixtures of the alkaline earths do not yield, as a rule, the characteristic forms above figured. The form of the oxalates separating from such solutions is then dependent upon the dominating element.

Since it is difficult to properly describe the peculiar changes to be observed, the student is advised to try the reaction on mixtures containing the elements of the calcium group, taking care to have first one, then another of these elements in slight excess.



1 Div. = 0.01 mm
Fig. 63.

When dealing with mixtures of *Ca*, *Sr*, and *Ba*, Behrens suggests the addition of a little acetic acid prior to that of the oxalic acid, then by cautiously neutralizing with magnesium carbonate, one element after another can be caused to separate. This method of procedure requires great care and considerable experience. For this reason it generally fails in the hands of the beginner.

III. Sulphuric Acid.

The method of procedure has already been thoroughly discussed. Attention has been called to the fact that from mixtures of the sulphates, *Ca* can

be extracted with hot water; *Sr* (and *Pb*) with hot hydrochloric acid; *Ba* remaining unacted upon.

Concentration of the water extract will give crystals of calcium sulphate.

Evaporation of the hydrochloric acid solution yields crystals of strontium sulphate.

The residual barium sulphate can be recrystallized from sulphuric acid, or can be converted into barium carbonate, dissolved, and tested.

With simple mixtures it is often unnecessary to proceed according to the above methods. Combinations of the different tests can be resorted to. For example, *Ba* can be precipitated in acetic acid solution by means of ammonium dichromate. The clear liquid is decanted, ammonium chloride and potassium ferrocyanide added, and the *Ca* precipitated and identified. The clear liquid is again separated and tested for *Sr* with sulphuric acid or potassium sulphate. The precipitated strontium sulphate can then be washed and recrystallized.

In addition to the above methods, it is possible to effect a fair separation by converting into nitrates, evaporating, and drying carefully. The perfectly dry nitrates can then be extracted with a mixture of absolute alcohol and ether. *Ca* nitrate is quite soluble, *Sr* nitrate much less so, while *Ba* nitrate is practically insoluble.

The alcohol-ether extract is evaporated to dryness, the residue dissolved in water, and tested for *Ca* with sulphuric acid, arsenic acid, or potassium ferrocyanide. The residual nitrates, insoluble in the alcohol, can then be tested for *Sr* and *Ba* by the dichromate method, or with stannic chloride and oxalic acid, or ferric chloride, sodium acetate, and oxalic acid; or oxalic acid in nitric acid solution.

In all cases the choice of method must be governed by the nature of the substance being examined. The ability to select, at once, the proper method of procedure which will yield the requisite information in the shortest possible time, and without error, is to be acquired only by experience and much practice.

E. M. CHAMOT.

Journal of Applied Microscopy and Laboratory Methods.

Edited by L. B. ELLIOTT.

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discontinue is sent.

ALTHOUGH the list of scientific journals and periodicals is already so long that it is quite impossible for one to gain even a casual review of the subjects they contain, the growing importance of investigation in which the principal feature is the collection of large series of statistical information has opened a new field, and it is proposed to establish a journal to be devoted to the publication of biological data and known as the *Journal of Biological Statistics*. Such a publication could certainly be made a valuable aid in the distribution of the results of research work.

There are at present no journals of biological science that wish to fill their pages, beyond a very limited degree, with long series of tabulated observations, which often form the basis of most important theories and conclusions. It is rather the rule to accept only the conclusions drawn by the investigator, and rely upon his judgment to interpret correctly the significance of the mass of facts he has collected. To be sure, this is necessary in most publications, as the great majority of readers cannot devote the time necessary to review carefully the ground covered. However, those studying the same or similar questions desire the most detailed reports of other workers in the same field.

It often occurs that men most capable of handling large series of statistics are not in position to collect them; and, on the other hand, men who collect data often fail to see the full significance of the facts before them. There are few men who, like Darwin, can collect facts and at the same time are able to give them the most accurate interpretation. It is therefore most desirable that data upon which theories and conclusions of general interest are based should have a medium through which they may have unlimited circulation.

* * *

The setting aside of the week in which January 1st falls, as a time for the session of scientific societies, will certainly receive general approval. It will undoubtedly be the means of increasing the attendance at the meetings. It will also certainly add to the good derived by those who go, for the heat of summer and the relapse that usually comes after the year's work naturally tend to lessen the enthusiasm of the members.

It is to be hoped that the universities and colleges throughout the country will coöperate in establishing the convocation week, thus making it possible for scientific men to assemble at a time favorable to the most profitable sessions.

* * *

Owing to the necessary insertion of other matter, the department of Laboratory Photography has been omitted from this issue.

CURRENT BOTANICAL LITERATURE.

CHARLES J. CHAMBERLAIN.

Books for review and separates of papers on botanical subjects should be sent to
Charles J. Chamberlain, University of Chicago,
Chicago, Ill.

REVIEWS.

Ikeno, S. Contribution a l'étude de la fécondation chez le *Ginkgo biloba*. Ann. Sci. Nat. Bot. Ser. VIII, 13: 305-318, pl. 2-3, 1901.

This paper contains a detailed description of fertilization and related phenomena in *Ginkgo*, from the formation of the ventral canal cell up to the

first division of the oöspore nucleus. The nucleus of the ventral canal cell rapidly disorganizes, while its sister nucleus increases in size and moves toward the center of the oösphere. In preparations stained with methyl blue and acid fuchsin, the metaplasmic ground substance of the nucleus stains red, and the chromatin, which forms a small, irregular, granular mass, also takes the red, while the nucleoli stain blue.

The nucleus now undergoes a great change in its structure, so that the metaplasm and chromatin can no longer be distinguished from each other. The further development of the nucleus of the oösphere agrees with the description of the corresponding phenomena in *Pinus laricio* as described by the reviewer some time ago. In one instance Professor Ikeno noted an abnormal development of the nucleus of the ventral canal cell, resembling the cases described for *Pinus laricio*.

The tube nucleus and the nucleus of the stalk cell disorganize within the pollen tube and do not enter the oösphere, and it is very probable that only one of the antherozoids is discharged, the other disorganizing without being able to enter. The nucleus of the antherozoid slips out from the cytoplasmic mantle before conjugating with the nucleus of the oösphere. The mode of fusion is like that already described for *Cycas revoluta*, i. e. the male nucleus gradually penetrates into the nucleus of the oösphere and lies within this nucleus before losing its own membranes. At the time of fusion the sex nuclei are very unequal in size, the female being about ten times as large as the male. The behavior of the chromatin during the fusion is not described.

The spindle in the first division of the fusion nucleus is very broad and multipolar and is never parallel with the longitudinal axis of the oösphere. In the case figured the spindle is tranverse. Fertilization takes place before the ovules fall from the tree.

C. J. C.

Gruber, Eduard. Ueber das Verhalten der Zellkerne in den Zygosporien von *Sporodinia grandis* Link. Ber. d. deutsch. bot. Gesell. 19: 51-55, pl. 2, 1901.

The zygosporangium of *Sporodinia* is surrounded by three coats, the outer of which is dark brown, warty, and cutinized, and is formed from the mem-

brane of the conjugating gametes, while the two inner coats belong to the zygosporangium itself. The middle coat is somewhat thickened and has a lamellate appearance, while the innermost is a mere Hautschicht.

Léger, who worked on *Sporodinia* six years ago, found that both gametes contain hundreds of small nuclei which become scattered in the mingling cytoplasm after the membrane separating the gametes has broken down. Double staining showed two kinds of nuclei, smaller ones near the periphery and much larger ones nearer the center. At a later stage, all the nuclei disappear and at each pole of the zygosporangium a spherical mass, the "embryonic sphere," is seen, each sphere containing a large number of granular bodies. These spherical masses increase in size and fuse with each other, and soon afterward numerous nuclei again appear, which pass into the germ tube as the zygosporangium germinates.

The present writer also finds a large number of nuclei in the zygosporangium, and finds that the nuclei are more numerous at the periphery, but there are also many nuclei in the center and all of the nuclei are approximately alike in size. This condition persists for a long time, and subsequent stages were hard to follow. No fusion division or disorganization of nuclei could be established with any certainty. On germination the nuclei appear in greater numbers in the germ tube. The presence of "embryonic spheres" is regarded as doubtful. Although the writer was not able to observe any fusion of nuclei, he believes that a fusion of the nuclei at the center of the zygosporangium is very probable.

C. J. C.

Davis, Bradley Moore. Nuclear Studies on Pelliæ. *Annals of Botany*, 15: 147-180, pls. 10-11, 1901.

This work was undertaken with the object of extending our knowledge of the cytology of the Hepaticæ, and with

the hope of throwing some light on the morphological relationships between the various manifestations of kinoplasm. Three phases in the life history of the plant were examined, namely, sporogenesis, the germination of the spore, and the vegetative activities in the seta. In the spore-mother-cell the spindles are developed in the same fashion as that which prevails in the spore-mother-cell of the Pteridophytes and pollen-mother-cells of Spermatophytes. In the stages of spore germination, asters with centrospheres were observed in the prophase. These, however, appear to be transitory structures as they disappear before the daughter nuclei are formed. In the vegetative cells the type of spindle formation is essentially similar to that described by Hof and Nêmec for the vegetative cells of the flowering plants. Davis also states that "it is probable, of course, that there is likewise a blepharoplast at the time of spermatogenesis." He concludes, however, that the kinoplasmic fibrillæ, the centrospheres and kinoplasmic caps are all secondary developments from the primal granular protoplasm, which is the only form of kinoplasm in any sense permanent in the cell.

Chicago.

A. A. LAWSON.

Noll, F. Ueber die Umkehrungsversuche mit Bryopsis, nebst Bemerkungen über ihren zelligen Aufbau (Energiden). *Ber. d. deutsch. bot. Gesell.* 18: 444-451, 1900.

In this paper Noll takes up again the much discussed subject of polarity among marine algæ. Beginning with the statement that in *Bryopsis muscosa*,

on which he worked, the polarity was as pronounced as in *Pinus*, he gives us some interesting results of his experiments; namely, that very few indeed of his plants reversed their root and shoot pole when inverted. Measurements and

dates show that the young and actively growing plants were so strongly polarized as to resume the original manner of growth; that only older, more slowly growing forms succumbed to the external conditions, and turned root into shoot and shoot into root. These results agree with those of Winkler of an earlier date.

Noll takes exception to the definition of "Energid" as given by Sachs, and calls the Siphonæ "single but multinucleate energids," laying stress upon the "Hautschicht" rather than upon the nucleus and its dominated mass of protoplasm. He therefore defines the energid as a "one or many nucleate plasmatic body enclosed in a definite wall."

PHILIP G. WRIGHTSON.

Chicago.

Life, A. C. The tuber-like rootlets of *Cycas revoluta*. Bot. Gaz. 31: 265-271, 10 figures in text, 1901.

The coral-like outgrowths on the upward rising roots of *Cycas revoluta* have long been known, and the endo-

phytic alga and fungi have also been described. Life has made a careful study of the subject from thin microtome sections and has been able to give a more precise account. In regard to the reputed dichotomy, he finds that not all of the meristem passes over into the two branches, but that a small portion is left as a bridge between them. This small portion, however, does not continue the main axis, and very soon disappears so that sections of roots in which the branching can be seen at the surface show no trace of meristem between the two branches.

The development of the algal zone is clearly figured and described. Three forms of fungi were observed. They make their appearance in advance of the algal zone and seem to prepare the way for the algæ, which are referred to the genus *Nostoc*. The presence of the fungi affects the intercellular spaces so that they become the rather large chambers occupied by the algæ. The *Nostoc* probably enters through the numerous lenticular areas. It is suggested that the tubercles serve for ærating and also assist in nitrogen assimilation.

C. J. C.

CYTOLOGY, EMBRYOLOGY, AND MICROSCOPICAL METHODS.

AGNES M. CLAYPOLE.

Separates of papers and books on animal biology should be sent for review to
Agnes M. Claypole, Sage College,
Ithaca, N. Y.

CURRENT LITERATURE.

Overton, E. Studien ueber die Aufnahme der Anilinfarben durch die lebende Zelle. Pringsheim's Jahrb. f. wiss. Bot. Bd. 34: 669-701, 1899.

The work gives the results of studies upon the action of anilin stains on animal and plant cells. Basic anilin stains are readily taken up by both

kinds of cells. Four classes of these stains were studied in detail. (1) *Triphenylenethane* stains: rosanilin (chlorhydrate, nitrate, sulfate), gentian violet, methyl violet, dahlia, anilin blue soluble in alcohol, toluidin blue, victoria blue, malachite green, methyl green, iodine green, auramin, rhodamin; (2) *Chinonimid*

stains: thionin, methyl blue, methylen green, safranin, toluylen red (neutral red), nigrosin soluble in alcohol, indulin; (3) *Azo* stains: chrysoidin, vesuvin, bis-marck brown, the last two being probably the same; and (4) *Acridin* stains: chrysanilin. All these stains penetrate the living cell most rapidly, only rhodamin is somewhat slower. Quite different is the effect of the sulphuric acid stains. (1) Acid fuchsin, acid green, acid violet, anilin blue soluble in water; (2) Nigrosin soluble in water, and indulin; (3) Congo red, ponceau red, bordeaux red, biebercher scarlet; and (4) Indigo carmin, all penetrate neither animal nor plant cells. Only the acid stains belonging to group three (*Azo* stains), methyl orange and tropäolin 00 and 000 are an exception, since these act in some cases after long immersion. Eosin and acid carmin are in general not taken up, curcuma acts quickly, carthamin more slowly. Since all the studies agreed that all the substances easily soluble in fatty oils and similar substances were taken up quickly by living cells, while those insoluble or soluble with difficulty did not penetrate living cells, the conclusion was obvious that the osmotic condition of living cells rests on the phenomena of selective solution. Especially was the author drawn to this conclusion since the plasma-skin of cells is impregnated with cholesterin or a mixture of cholesterin and lecithin. It especially concerns these anilin colors, since all the commercial salts are basic anilin stains mixed with cholesterin, or else are easily soluble in a strong solution of cholesterin or lecithin in organic fluids. Also these liquids in a pure condition have no solubility for these dyes. Tannic acid-methylen blue, which does not penetrate the living cell, is also wholly insoluble in cholesterin and lecithin solutions. With a few exceptions all the sulphuric acid stains and acid carmines are completely insoluble in these liquids. Methyl orange and tropäolin are exceptions and penetrate very slowly and slightly into the living cell.

A. M. C.

Retterer, E. Transformation de la cellule cartilagineuse en tissu conjunctiv réticulé. Comp. Rend. Soc. de Biol. 51: 904-907, 1899.

For this work sublimate solution, Zenker's and Flemming's fluids and also an aqueous solution of platinic chloride, 1 pt. to 1000, were used. Without previous decalcification, objects such as the ribs of rabbits and guinea pigs, are embedded in paraffin. The following combination of stains gave the best results: after leaving the sections for a few hours in a solution of safranin in anilin water, they are washed out in water, stained for a few minutes in Boehmer's hæmatoxylin, and decolorized in alcohol to which a very little picric acid is added.

A. M. C.

Petroff, N. Neue Färbungsmethod zur rothe Blutkörperchen in Schnittpreparaten. Bolnicznaja Gazeta Botkina, 1899. (Russian.)

Up to now the contrast-staining of blood corpuscles has been done by the use of stains from the malachite-green group, which differentiate by virtue of the special characters of red blood cells. This process is as follows: material previously fixed in Müller's fluid or formalin, or Orth's mixture, is embedded in paraffin, not collodion, cut into the thinnest sections possible of regular thickness, and fastened to the slide. The paraffin is then taken out with xylol and the sections washed in alcohol and water. (2) Nuclear staining is done by putting the sections for 10-15 minutes in a concen-

trated solution of bismarck brown in one per cent. acetic acid, or in lithium or borax carmin for 20–30 minutes; in case of using borax carmin it should be washed out in hydrochloric acid alcohol. Washing with water follows. (3) Stain next for 10–15 minutes with 20 per cent. aqueous solution of malachite green, also brilliant green or victoria green. The solution is made by diluting the alcoholic solution five times. Wash out in water. (4) Stain for $\frac{1}{2}$ –1 minute long according to Van Gieson's tincture method or with concentrated aqueous picric acid solution, which is diluted 4–5 times with water. Wash in water. (5) The quickest possible dehydration and decolorization in absolute alcohol, mounting in xylol and balsam. Turpentine or bergamot oil may be used in the place of xylol. All the decolorizing necessary is easily managed, and can be allowed to continue for a long time. In preparations made in this way the beryl-green corpuscles are distinguished from all other structures, which are a gold-brown from bismarck brown or red-gold from carmin.

A. M. C.

Godlewski, E. O. rozmnozaniu jader w nies-
niach prazkowanych zwierzat kregowych
(Ueber Kernvermehrung in den querges-
treiften Muskelfasern der Wirbelthiere.).
Bull. de l'Acad. des Sci. de Cracovie, Avril,
1900.

In order to learn to recognize the multiplication of nuclei in striated muscle of vertebrates during embryonic and postembryonic development, the author studied the striated muscle of

newly born guinea pigs and mice, also those of salamander larvæ. The extremities of embryos taken from the mother or of narcotized newly born young were put in toto into the fixation fluid. Perenyi's fluid or concentrated sublimate solution with the addition of two per cent. acetic acid was used, followed by increasing strengths of alcohol. After hardening, small pieces of muscle were separated from the bone. During these fixing and hardening processes a great deal of contraction takes place in the muscle fibers. Muscles were cut in paraffin in longitudinal, transverse, and oblique sections five μ in thickness. These were stained in thionin, also in Heidenhain's hæmatoxylin, double stained with bordeaux red or eosin. In preparations so made the nucleoli are sharply tinted with red, so that a clear contrast is obtained between these and the blue chromatin bodies.

A. M. C.

His, W. Ueber Sogenannte Amitosis. Anat.
Anz. Centralblt. f. d. Gesam. Wiss. Anat.
18: 52–60, 1900.

Since the discovery and demonstration of bipolar mitosis it has been known that nuclear figures exist which do not

agree with the newly discovered principles. Flemming gives a second type of division, direct or amitotic. The characters of this kind are negative, absence of the spindle and splitting of the chromosomes. This form was considered degenerate or pathological, but recent work shows that by changing the conditions of growth the cells of *Spirogyra* may be made to pass from mitotic division to amitotic and back again, without disturbing the normal conditions of growth. This places the difference in the two types of division in the province of physiology and the problem is to determine in how far the two processes follow a common law, and in what way they are related to each other. It has already been suggested (His) that amitosis may be plenipolar mitosis and be related to the growth of multinuclear giant cells and syncytial formation. For these processes His suggests the name "syncaryosis."

In the division of the periplast cells of Selachians two types are recognizable, one in which the nuclei have central, regularly arranged chromatin, and another in which the chromatin is rod-shaped in separate pieces. Those of the first type are found earlier in development. The nuclei contain several small granules to which small furrows run radially. These granules grow to large masses without losing their relations to the radial grooves. Later a giant cell contains six, eight or more large nucleoli. The nuclei of the second type with separate chromatin rods are distinguished by their transparency and even staining. Transition forms are found forming two lines, one in the direction of dissociation and one towards synthesis. The former process is a simple breaking up of the chromatin rods into fine granules. Many stages of these are found. Reconstructive processes follow definite steps: (1) Breaking up of the plasma bodies, carrying the chromatin into several small balls; (2) separation of these balls, still remaining connected by a thread; (3) re-appearance of chromatic rods; (4) radial structure appears in connection with chromatin; (5) formation of enclosed nuclei, thickening of nuclear wall. The process continued still farther and showed itself that of a syncytial formation; it is a kind of nuclear division. Comparing this process with regular bipolar mitosis, we find in common the phases of dissociation of chromatin—prophases; the formation of the chromatin rods and their radial arrangement are the anaphases. The metaphase would correspond to the dissociated mass of granules. As long as the plasma of the nucleus retains a connection with the dissociated chromatin a “spirem” is present. The equivalent of spindle fibers are the plasma threads stretched between the nuclear balls. The origin and relations of polycentric giant cells are understandable on general cellular laws. It is known that a central force acts in such phenomena as division. Its nature is unknown, but simple exhibitions of “pull” and “push” are to be seen.

The process of mitosis can be divided into five steps: (1) The division of the pre-existing centrosome; (2) the separation of these parts; (3) the changed influence of these centrosomes, due to their changed position, shown in the appearance of double radiations; (4) the grouping of the chromatin bodies and arrangement in the daughter nucleus; (5) the formation of cell walls. Each of these processes requires a separate time; but any change in the time requisite for these steps may change the appearances entirely. If the division of centrosomes is relatively too rapid, new ones arise without the correlated changes, and the subsequent steps are those of cells with many centrosomes. The distribution of the chromatin is, hence, difficult to follow. The relation of the nucleolus to these centrosomes and the plasma cells remains yet to be studied.

A. M. C.

Moll, A. Zur Histochemie der Korpels. From the results of the author, Tanzer's Centralbl. f. Physiol. 13: 225-226, 1899. orcein solution (orcein 0.5 gram, alcohol absolute 40.0 c. c., dist. water 20.0 c. c., hydrochloric acid 10 drops) is an instructive double stain for embryonic cartilage. The preparations (embryos or parts of them) must be hardened in alcohol (not in chromic acid), and then in thin celloidin sections be put into the above staining solution for 6-24 hours, then washed in 80 or 90 per cent. alcohol until the celloidin is nearly colorless,

dehydrated in ninety-eight per cent. alcohol, cleared in origanum oil, and mounted in balsam. All preformed hyaline cartilage shows itself distinct microscopically, through its blue violet color, from the rest of the brownish red tissue. Microscopic investigation shows that the blue color has its place in the ground substance of the cartilage. This blue cartilage network makes a strong contrast with the red nuclei of the merely light blue or non-colored cartilage cells. In the embryonic fibro-cartilage of the intervertebral discs, as yet undifferentiated, the central cartilage cells stain blue, the nuclei red. The cells always become paler toward the margin. With orcein, embryonic elastic cartilage gives no double stain. The change in the color from that of adult cartilage is worth mention. Here the ground substance is reddish, the cartilage cell with its surrounding area intense blue, so that the red nuclei can only be seen in the thinnest sections. Similarly changed is the adult fibro-cartilage; only a few fibro-bundles are blue. The elastic cartilage also shows no double stain in the adult condition. Developed bone, both decalcified and non-decalcified, likewise shows no double stain.

E. J. C.

Linser, P. Ueber den Bau und die Entwicklung des Elastischen Gewebes in der Lung. Anat. Hefte. H. 42, 43: 307-336, m. 3 Tfn., 1900.

The Weigert method was used to demonstrate the elastic tissue in the lung.

The stain acted usually in 2-3 hours, yet a longer time for staining, even to 24 hours did not do much harm. If a shorter time was used, 15-20 minutes, no usable results could be obtained. By the longer staining one had this advantage, with others, to stain the adjoining kinds of connective tissue in contrast. Usually a simple washing out in strong alcohol sufficed to make the bundles appear separate. After a longer continuance of the staining process it is necessary to differentiate in hydrochloric acid alcohol if one wishes to have the elastic fibers stained. For a nuclear stain, alcoholic borax carmin and lithium carmin were used; control-stains were carried on with hæmalum-eosin after Van Gieson's method. For investigation 12 embryos, 3.3 cm. long (Kopf Steiss), to the oldest foetal stages, were used. Further, fourteen children up to five years of age and eight older human lungs of different ages. Further, eight different stages of the rat, both before and after birth, lungs of young and old cattle, of new born and older guinea pigs, of hare, dog, horse, pig, roe, stag. Tissues were preserved in formalin or alcohol and imbedded in celloidin.

E. J. C.

Foote, K., and Strobell, E. C. Egg of *Alloboophora foetida*. Journ. of Morph. 16: 607-618, 3 pls., 1900.

A series of micro-photographs of this egg is published to illustrate the following points: (1) The effect on the

cytoplasm of the different fixation fluids now in common use. (2) The character of the fertilization cone. (3) The position of the middle piece of the male aster. (4) The origin of the sperm granules. (5) The early stages in the development of the pronuclei. (6) The presence of osmophile granules in the nucleoli of the germinal muscles. The photographs have been taken at two magnifications, 660 and 950, and it is believed that proof has been offered of some of Foote's earlier conclusions, in regard to the cytoplasmic origin of centrosome of the male aster.

A. M. C.

CURRENT ZOÖLOGICAL LITERATURE.

CHARLES A. KOFOID.

Books and separates of papers on zoölogical subjects should be sent for review to Charles A. Kofoid, University of California, Berkeley, California.

Fülleborn, Dr. Ueber Formalinconservierung. After several years' experience in collecting in temperate regions and the tropics, Dr. Fülleborn gives his unqualified approval of formalin as a preservative of zoölogical material. Its portability, cheapness, ease of application, and its qualities as a preservative for histological purposes, combine to commend it for use in preference to alcohol on collecting expeditions. Large objects for anatomical work should be hardened in 5-10 per cent. formalin for 8-14 days. For transportation, objects thus hardened may be packed in excelsior moistened in formalin, in zinc cases which are soldered up when filled. These zinc cases are made up in sizes which "nest" readily for transportation into the field. Large fish should be opened along the ventral side and along the vertebral column, or the skin should be freed from the musculature in places and a wadding saturated in formalin thrust beneath it. Small fish may be thrown into the formalin solution or injected in the digestive tract. Formalin is especially recommended for fish whose scales are easily rubbed off. Large fish kept for six years in formalin, in relatively weak solutions, are still in a state of excellent preservation.

As a preservative of natural colors, formalin has not fulfilled the high hopes which it first called forth. Dr. Fülleborn reports that it preserved color well in some tropical Amphibia, and in many other instances specimens reached European museums from the tropics in unfaded condition. On the other hand, the iridescent colors of fishes fade as quickly in formalin as they do in alcohol. The brilliant pearly sheen found on certain beetles was preserved in specimens in formalin, though it faded at once in dried and in alcoholic material. The egg-masses of *Necturus* with their gelatinous coverings have kept well in formalin, the form, the eggs, and transparency of the membranes remaining unchanged. Tropical plankton was preserved in 2-5 per cent. formalin, the algæ retaining the green color of the chlorophyll and the smaller *Entomostraca* keeping their natural form as a rule. Some species, however, are distorted by the formalin.

Small birds were mummified by injecting a solution of 5-10 per cent. formalin saturated with sodium arsenate with a hypodermic syringe into the thoracic and abdominal regions, the musculature of the breast and shoulders, the eyes, and the brain (through the orbit). Injections should not be made between the musculature and skin. The small openings made by the syringe do not permit the fluid to escape and soil the feathers, if care is taken in handling the birds. This fluid is to be preferred to 15 per cent. carbolic acid, sometimes used in mummifying birds, since it does not destroy the color of the feathers wet by it. Large birds may be treated (in addition to the injection) by removing the viscera and packing the body cavity with wadding saturated with the injecting

fluid. After injection the feathers should be properly arranged and the birds hung by the bills, when they will dry rapidly. Specimens thus treated may be softened subsequently and mounted in the usual manner. This method is not only a rapid one, facilitating field work, but it also preserves the skeletons.

C. A. K.

Saint-Remy, G. Contributions a l'étude du développement des Cestodes. Arch. de la Parasit. 3: 292-315, pl. 7, 1900.

The small size and the very resistant membranes of the eggs of tapeworms render the technique of their study by modern methods a matter of considerable difficulty. These difficulties have been surmounted to some extent by Professor Saint-Remy, who has studied the development of two species of *Anoplocephala*, parasites of the horse. After removal from the host, the worms were kept in normal salt solution. Examination of the living eggs reveals but little, and the study of sections of the proglottids for the development of the eggs contained therein is even less satisfactory. The eggs are freed from the worm by compression or laceration, and are collected upon slides in sequence from the last proglottid, forward as far as they can be found, thus securing successive stages in development. The eggs are killed upon the slide, and the coagulated fluid in which they lie serves to fix them to the glass. A large number of reagents were tried, and good results were obtained with the aqueous solution of corrosive sublimate, and also with Carnoy's fluid (absolute alcohol 6 vol., chloroform 3 vol., glacial acetic acid 1 vol.). The eggs were not sectioned, but were mounted *in toto* in balsam. For this purpose it was found that alum-carmin and also *bleu de toluidin* eosin gave the finest results.

The development of *Anoplocephala* resembles that of *Tænia*. A small egg-cell and a large yolk mass are enclosed in the egg shell. The former gives rise to two minute polar cells. Two of the cleavage cells invade the yolk, grow at its expense into two giant cells which form the outer covering surrounding the embryo, which is ultimately cast off. Three or four other cells form a second envelope, a pyriform cap provided with branching filaments in the form of a grapnel, and the balance of the embryonic mass forms the onchosphere or embryo proper, within which the characteristic hooks are formed before the embryonic membranes are shed. No decisive evidence is contributed to the solution of the problem of the germ layers in Cestodes.

C. A. K.

Cope, E. D. The Crocodilians, Lizards, and Snakes of North America. Report U. S. Nat. Mus., 1898, 153-1270, 36 pls. with 347 figures in the text, 1900.

Students of our native reptiles will welcome this posthumous work of Professor Cope, for it is a very comprehensive manual, including all of the nearctic species of the orders Loricata and Squamata. It is based upon the extensive collections of the author, of the Philadelphia Academy of Natural Sciences, and the U. S. Natural Museum. While it deals mainly with the taxonomy of the group, it also gives many facts pertaining to the external and internal anatomy, especially the osteology of the species described. Incidentally reference is made to many interesting points in the biology and natural history of the animals discussed. Ample synoptic keys are provided for the purposes

of identification of species, while abundant illustrations facilitate the recognition of diagnostic characters. The fact that this monograph is issued in the Report of the U. S. National Museum makes possible a much wider distribution to the public than was given Professor Cope's earlier bulletins upon the Batrachia. It is to be hoped that the monograph of the turtles, in preparation by the late Professor Baur, will soon be issued to complete the manual of the North American Reptilia.

C. A. K.

Sayce, O. A. A Method of Preserving Crustacea. Victorian Nat. 17: 73-78, 1900.

Suppleness in dried specimens of such animals as the Crustacea is a great desideratum, especially in laboratory demonstrations. Mr. Sayce secures this and also preserves to a considerable degree the natural appearance of the animal, and at the same time obviates preservation in fluids, by the following treatment: The specimens, either fresh or from 70 per cent. alcohol, are immersed for some days, ten will suffice for crayfish, in a fluid which, in metric equivalents, has approximately this formula:

Glycerin	- - - - -	375 c. c.
Methylated spirit	- - - - -	250 c. c.
Water	- - - - -	250 c. c.
Corrosive sublimate	- - - - -	0.5 gm.

Slight punctures in inconspicuous parts of the carapace will facilitate penetration. After thorough soaking in this fluid, the specimens are removed and drained and allowed to dry. They can be stored in boxes or wrapped in waterproof paper. To avoid too much drying and also to prevent the accumulation of moisture due to hygroscopic action of the glycerin, specimens should be given a thin coat of gelatin and then immersed in 10 per cent. formalin for a few minutes. This hardens the gelatin, renders it impervious to water, but does not interfere with its transparency.

C. A. K.

NORMAL AND PATHOLOGICAL HISTOLOGY.

JOSEPH H. PRATT.

Harvard University Medical School, Boston, Mass., to whom all books and papers on these subjects should be sent for review.

Fujinami. Ueber die Beziehungen der Myocarditis zu den Erkrankungen der Arterienwandungen. Virchow's Archiv., 159: 447-490, 1900.

The circumscribed areas of acute parenchymatous myocarditis are always associated with the narrowing or occlusion of the small arterial branches

which supply them. This is the only form of myocarditis which bears a close and constant relation to sclerosis of the coronary arteries. In fibrous myocarditis sclerotic changes are found in the course of the coronary arteries, but not usually in immediate connection with the fibrous areas.

Arterio-sclerosis, without complete occlusion of the vessels, leads to disturbances of nutrition in quite large portions of the muscle-wall. Degeneration of the muscle-fibers results, followed by a reparative growth of connective tissue.

A destruction of the muscle does not always take place. The author describes a primary interstitial non-purulent form of myocarditis in which collections of cells pushed aside the intact muscle-fibers. He regards this variety as toxic in origin. The foci of cells are at length replaced by fibrous nodules.

The seat of the disease in the blood-vessels can be in the main branches of the coronary arteries, or outside the heart in the root of the aorta, or in the orifices of the coronary arteries.

Fujinami concludes that fibrous myocarditis originates in a variety of ways. It is simply the final outcome of a number of different pathological processes. The thickenings of the vessel-walls, demonstrable microscopically, are not always to be regarded as the cause of the formation of the fibrous areas. The vascular changes can occur as a result of the fibrous myocarditis just as the vessels in the scar tissue of healing ulcers become sclerosed.

Fragmentatio myocardii is frequently associated with sclerosis of the coronary arteries and fibrous myocarditis.

J. H. P.

Warthin. Accessory Adrenal Body in the Broad Ligament (Adrenal of Marchand). *American Journal of Obstetrics*, 42: 797-805, 1900.

According to Schmorl, accessory adrenals are found in the neighborhood of the adrenals, in the adrenal and solar plexuses, and along the adrenal

and spermatic veins, in 92 per cent. of all autopsy cases. Accessory adrenal tissue has been found in the kidney capsule and cortex. Along the spermatic vein, in the pampiniform plexus, between the testis and epididymis, in the corpus Highmori, pancreas, liver, and broad ligament.

The author was able to collect from the literature only 23 cases of accessory adrenals in the broad ligament. Marchand in 1883 was the first to report this anomaly. The diagnosis is made by the characteristic epithelial-like cells, and the relation of these cells to the connective tissue and capillaries. Usually the structure of the body is uniform throughout, in some cases resembling the cortex and in others the medullary portion. As a rule the accessory adrenals of the broad ligament consist of cortical tissue only. The accessory adrenal found by Warthin was a pale yellow, fat-like body of the size of a pea. It was situated behind the ovary, near the plexus of veins.

J. H. P.

Abbott, M. E. Pigmentation Cirrhosis of the Liver in a Case of Hæmochromatosis. *Journal of Pathology*, 7: 55-69, 1900.

An advanced cirrhosis of the liver and a moderate degree of chronic interstitial pancreatitis were associated with

an extensive deposit of iron-containing pigment in the tissues. There was a bluish gray slaty tinge of the skin, and a rusty brown discoloration of the internal organs. Sections of the liver and pancreas were loaded with golden-brown pigment, responding with a deep blue color to Perl's test for iron, which was present also, though in a lesser degree, in the spleen, suprarenals, and heart muscle. There was more or less fibrosis of all the organs except the kidney. In both liver and pancreas the heavy pigmentation of the connective tissue had its source, in part at least, in the broken-down pigmented cells of the parenchyma. A fairly advanced chronic interstitial pancreatitis existed without the clinical picture of diabetes so common in cases of advanced hæmochromatosis.

Sections of the organs were tested for iron with ammonium sulphide and with potassium ferrocyanide, with affirmative results. In the closer study of the case Perl's test only was used. The routine method at first employed was as follows: Potassium ferrocyanide, 2 per cent. solution, three minutes; hydrochloric acid 1 per cent. watery solution, two to five minutes; wash with distilled water. The bulk of the material was hardened in Müller's fluid to which 2 per cent. formalin had been added, and was preserved in methylated spirits. With the fresh tissue the reaction was prompt, but after two months no typical reaction occurred, the granules turning green or a greenish yellow; many did not react at all. That the iron was not liberated, but that the reaction was only delayed, was proved by the fact that sections left in the hydrochloric acid solution, two to twenty-four hours, gave a typical Prussian blue color, while when the test was performed with hot hydrochloric acid the reaction was almost instantaneous. Sections kept in 4 per cent. formalin gave a typical reaction in two minutes with cold hydrochloric acid. Bits of tissue hardened in alcohol reacted readily. Müller's fluid seems thus to have been the cause of the delayed iron reaction.

Four other cases of hæmosiderosis were studied by the author. She concludes that in general hæmachromatosis some primitive agency, as yet unknown, is at work leading to (a) an increased destruction of hæmogloin taking place either in localized hæmorrhages, or within the blood stream, or perhaps sometimes within the parenchymatous cells themselves; (b) a degeneration of the cells of certain organs by which they become unable to throw off the granular pigment deposited in them, and, becoming loaded, finally disintegrate. The cirrhosis would seem to be the nature of a chronic interstitial inflammation, secondary upon the presence in the tissues of pigment set free after the destruction of the parenchymatous cell.

J. H. P.

GENERAL PHYSIOLOGY.

RAYMOND PEARL.

Books and papers for review should be sent to Raymond Pearl, Zoölogical Laboratory, University of Michigan, Ann Arbor, Mich.

Mühlmann, M. Über die Ursache des Alters. Grundzüge der Physiologie des Wachstums mit besonderer Berücksichtigung des Menschen. Wiesbaden (J. F. Bergmann), pp. xii und 195, mit 15 Abbildungen, 1900.

This discussion of the general physiology of growth begins with a comparison of the biological relations of unicellular and multicellular organisms. From the two

premises that, on the one hand, the differences between unicellular and multicellular organisms are the results of the close proximity of the cells to one another in the latter as compared with the former, and, on the other hand, that one most important difference between the two is that the multicellular organism dies, the author arrives at the conclusion that *growth causes death*. This preliminary statement of the general standpoint opens the way for an analysis of the laws of growth. The subject is developed in the following way: Growth is primarily

related to nutrition. In the case of the Protozoa each cell is able to take nutriment and carry on respiration over its whole surface. When, however, the cells resulting from the division of a single one remain permanently in contact with one another, it necessarily follows that a smaller portion of each cell can come into contact with and absorb nutriment. Such developmental forms as the blastula and gastrula, and in fact all folds, furrows, invaginations and evaginations appearing during development, are explained as the result of the insufficient nourishment which is unable to keep pace with the growth. Since only the cells immediately in contact with nutritive substances are sufficiently nourished to support growth, while the cells within and away from nutriment are correspondingly insufficiently nourished, these latter very soon begin to degenerate and show necrobiotic phenomena.

The author considers that practically all processes of cell differentiation are, from the standpoint of the cell, regressive in nature. The primitive cells or "Blastzellen," from which are developed all other kinds of cells, are seen in the embryo before any beginning of differentiation, and are characterized by their large size, their richness in cytoplasm, and their large nuclei. Such cells are also found in the adult body in the Malpighian layer of the epidermis, the mucous lining of the alimentary canal, the endothelium of the blood vessels, the germinal epithelium, the osteoblasts, etc. All *changes* which occur in these "Blastzellen" are regressive in nature. The only progression is found in their *multiplication*, which is possible up to a very old age.

The theory is next applied in detail to the processes of ontogeny and histogenesis. It is believed that the development of the individual begins with the formation of the ovum within the ovary. The maturation of the ovum marks the beginning of the degenerative changes which ultimately lead to the death of the individual. The egg is left poorer in protoplasm and nuclear material after maturation. The development of the body form in all its details is explained as a result of the better nutritive conditions of cells on the periphery over those in the center of an embryo or an organ. The same principle is applied to the differentiation of the various tissues. Muscle cells or ganglion cells are degenerated because they have lost the cuboidal or polygonal form of the "Blastzellen" and are less rich in the sort of protoplasm that makes up the body of *Amœba*. The chemical as well as the morphological aspect of histogenesis is developed.

The relation of function to structure and the origin of the functional differentiations and adaptations are next discussed. The author is strongly opposed to a teleological consideration of life phenomena and in order to escape some of the difficulties along this line which his theory involves, he advances some astonishing physiological principles. An example will indicate the nature of these. It is stated that the saliva is a product of the regressive metamorphosis of the poorly nourished cells of the salivary gland, and is *useless* to the organism. The ptyalin is useless because the sugar into which it converts the starch of the food has to be reconverted into "animal starch."

The remainder of the book is devoted to a collection of data relating to the growth in size and weight of the human body and its organs, from birth to old age. These data support the author's view that there is during the course of

life a steady diminution in the relative amount of the additions to the size and weight of the body in successive years after birth. The same is true of the organs and tissues except in the case of those which are largely made of "Blastzellen." Such tissues, as for instance the epithelial lining of the alimentary canal, continue to grow by the multiplication of cells until nearly the time of death. The reason for the greater absolute weight of an adult man over that of an infant is found in the fact that the muscular and skeletal systems take on weight by processes of cell metamorphosis essentially regressive in character, there being in these cases no growth by cell multiplication after early life.

The work is one of value on account of the mass of data on the growth of the human body which it presents. While it is probable that few would agree with all the theories proposed, the discussion nevertheless brings out strongly the possible importance of cell nutrition as a factor in developmental processes.

R. P.

Bataillon, E. La pression osmotique et les grands problèmes de la Biologie. Arch. f. Entwick.-mech, II: 149-184, pl. 5, 1901.

The general standpoint of the author is that osmotic pressure is a general and fundamental factor in biological phenomena, and should furnish the basis for the investigation of such important problems as the resistance of organisms to dehydration (latent life,) teratogeny, the production of multiple embryos, and artificial parthenogenesis. On all these points experimental results are offered. The first experiments discussed are on the extraordinary ability of *Ascaris* eggs to resist the action of fixing agents and other poisonous fluids. The reasons for this resistance capacity are found in the facts that the egg is surrounded by a membranous chorion which is semi-permeable, and that the fluid of the interior of the egg is of such a concentration as to furnish a very high osmotic pressure. On account of this high osmotic pressure ordinarily harmful substances cannot enter the egg. There is no plasmolysis of the egg in the fluids of less osmotic pressure than that of a 15 per cent. solution of NaCl. The eggs are unable to withstand the dehydration produced by a 30 per cent. solution of NaCl. The author thinks that cases of "latent life," of which desiccated rotifers form a good example, are to be explained as a result of the great osmotic pressure of their body substance which resists the extraction of water beyond a certain point.

Loss of water is found to have a retarding influence on development and may completely stop it. The eggs of *Petromyzon Planeri* show no segmentation in a 1 per cent. solution of NaCl, while in a .2 per cent. solution their development proceeds normally. In solutions of intermediate concentrations there are varying degrees of retardation. Solutions of CaCl_2 and sugar isotonic with the NaCl were tried and the same results were obtained, indicating that the osmotic pressure is the important factor rather than the chemical composition. Twin larvæ of *Petromyzon* were obtained by placing the fertilized eggs for a certain time (about eighteen hours) in solutions isotonic with 1 per cent. NaCl and then removing them to water, in which the development took place. Fertilized eggs of the teleost *Leuciscus rutilus* treated in the same way (except that they were kept in the solution only one hour) developed into multiple monstrosities.

By the use of the same method, with variations in the time of action of the solution, the author obtained segmentation of unfertilized eggs of some fish and

amphibians. *Leuciscus rutilus* and *Rana esculenta* gave the best results. The explanation offered for all of these phenomena has its basis in the osmotic effect of the different solutions.

R. P.

Kijanitzin, J. J. Weitere Untersuchungen über den Einfluss sterilisirter Luft auf Thiere. Virchow's Archiv, 162: 515-533, Taf. 14, 1900.

In a series of papers which have appeared at intervals during several years past, Kijanitzin has given an account of his experiments on the physiological effects of sterilized air on animals. The paper here considered sums up the results which have thus far been gained and announces a conclusion which, if proven by future investigation to be true, will be of great significance with reference to the general subject of animal metabolism. The author maintains that in addition to the oxygen of the air there are necessary for the normal metabolism, and consequently the life of the animal, certain micro-organisms. These micro-organisms, entering into the blood in the process of respiration, are taken up by the leucocytes and digested. In the course of their digestion an oxydising enzyme is given off. By the action of this enzyme under normal conditions oxidation processes in the tissues are brought about. The experiments seem to show that in the absence of this enzyme the normal process of oxidation in the animal quickly declines, and soon ceases altogether. The animal then dies on account of the formation of large quantities of incomplete, intermediate products of metabolism (leucomaines).

R. P.

Weinland, E. Ueber den Glykogengehalt einiger parasitischer Würmer. Zeitschr. f. Biol. 41: 69-74, 1901.

In an analysis of specimens of a tapeworm (*Tenia expansa*) and of the parasitic nematode *Ascaris*, Weinland found a very high glycogen content in both cases. In *Tenia* the glycogen amounted to 1.5-4.7 per cent. of the fresh animal, while in *Ascaris* the amount was 4.2-7.1 per cent. of the fresh animal. The amount of glycogen in the dry substance was found to be in the case of *Tenia* 15-47 per cent, while in *Ascaris* it was from 20-34 per cent. The highest previously known glycogen content was in the mussel *Cardium*, 14 per cent. of the dry substance of which is glycogen. In mammals the glycogen content is rarely more than 3 per cent. of the dry weight. The author discusses the chemical nature of the glycogen obtained from these worms.

R. P.

Macy, M. L., and Norris, H. W. A General Physiology for High Schools, Based upon the Nervous System. pp. 408. (No date.) American Book Company, New York.

In this text-book the authors have endeavored to bring all the conventional subject matter of the "high school physiology" under one point of view, and so treat its individual phases in their relation to a common basis. The idea is commendable, but the choice of a basis, or view point, is not altogether fortunate. The attempt is made to discuss *all* the structures and activities of man's body as things primarily related to the nervous system. It will readily be seen that such a method is a purely artificial one, and, from a physiological standpoint, unsatisfactory. The detailed treatment of most of the topics is very good. The most excellent features of the work are the sections devoted to laboratory and demonstration methods for the use of the teacher. Some of the methods of

demonstration with simple apparatus are ingenious and valuable. Especially worthy of mention in this connection are the methods given for illustrating the processes of circulation and respiration. The text figures are numerous and for the most part copied from standard works. As a whole the book makes a very good impression and, in the hands of a competent teacher, ought to prove an excellent high school text.

R. P.

CURRENT BACTERIOLOGICAL LITERATURE.

H. W. CONN.

Separates of papers and books on bacteriology should be sent for review to
H. W. Conn, Wesleyan University, Middletown, Conn.

Migula, W. System der Bakterien. Vol. II. A second volume of Migula's System der Bakterien has made its appearance. The author's original purpose was to collect all species of bacteria which had been described, and, by testing them in culture media in his own laboratory, to make comparative studies and descriptions. This task proved to be wholly impracticable. Many of the species he could not obtain, and many of those sent him were not in condition for study. The book is therefore simply a compilation of descriptions of species as given by the original authors. It is a large work of 1068 pages, with 35 figures, and indicates an immense amount of labor in compilation on the part of the author.

H. W. C.

Beijerinck. Anhäufungsversuche mit Ureumbakterien. Cent. f. Bak. u. Par. II, VII, p. 33, 1901. The very great importance of the fermentation of urea makes it somewhat strange that the bacteria producing this phenomenon have not been more carefully studied. Until the appearance of this work of Beijerinck very little has been known in regard to the micro-organisms concerned in urea fermentation, a few observations made some time ago comprising our sole information. The author, however, has investigated the subject, and has described, with excellent figures, five new species of micro-organisms associated with this universal and significant fermentation. These specimens include bacilli, some of which have flagella and others not, and it also includes a *Sarcina* species which is motile and abundantly provided with flagella; a somewhat unusual relation. The author also studied the subject from a physiological standpoint and concluded that the decomposition of urea is produced by an enzyme, *urase*. This enzyme is completely insoluble, and is so intimately bound to the body of the bacterium that it cannot be separated from it.

H. W. C.

Stutzer. Die Organismen der Nitrifikation. Cent. f. Bac. u. Par. II, VII, p. 168, 1901. For some years there has been a dispute between Stutzer and the Russian bacteriologist, Winogradsky, in regard to the nature and the physiological properties of the extremely important soil organisms known as *nitrifying bacteria*. Winogradsky, who originally discovered them, has described them as bacteria having a most extraordinary sensitiveness to the presence of organic substances,

being prevented from growth by the presence of the smallest amount of organic material or ammonia. In previous work Stutzer has taken grounds quite in opposition to many of the points which were held by Winogradsky. The present article is practically a withdrawal on the part of Stutzer of all of his previous claims. In an introduction he explains how, in the press of engagements, he was led into error by leaving the work to an assistant, and he now reports the results of more careful work. In practically all respects Stutzer now agrees with Winogradsky, so that the relation of the nitrifying bacteria to the various conditions of nature which had previously been so carefully described by Winogradsky, must be taken as confirmed by this latter work of his opponent Stutzer. Stutzer studies both the *nitrate* and the *nitrite* forming bacteria, and in most respects comes to identical conclusions with those of the Russian bacteriologist.

H. W. C.

Hiltner. Ueber die Ursachen, welche die Grösse, Zahl, Stellung und Wirkung der Wurzelknöllchen der Leguminosen bedingen. Cent. f. Bak. u. Par. II, VII, p. 202, 1900.

As is well known, all species of legumes growing in ordinary soil are likely to develop tubercles on their roots through the agency of bacteria. Out-

side of the family of legumes only three or four families of plants are known to produce similar tubercles, and these only in exceptional cases. The author raises the question as to the reason why the soil bacteria have this power of growing in the roots of legumes. Thinking that it was possible that the organisms produce some secretion which affects the roots of the legume, he instituted experiments, the result of which was to show him that: (1) the changes in the root hairs of the legumes accompanying the production of a tubercle are produced by some soluble substance secreted by bacteria; (2) this substance is present in great quantity in the tubercles; (3) the older root hairs are immune against the action of this substance.

The author finds that different cultures of the tubercle organisms have considerable difference in their power of producing tubercles. When a plant which already possesses tubercles is inoculated with culture of a bacteria of a higher virulence, it is noticed that there is a very considerable increase in the number and size of the tubercles. If, however, a plant possessing tubercles is inoculated by a culture of the same virulence there is no increase of number of tubercles. In other words, according to the author's conclusions, the presence of the tubercles renders the legume immune against the further action of cultures of the same grade of virulence, although they are not immune against a culture of a higher virulence.

H. W. C.

Plorkowski and Jess. Bacterium coli als Ursache eines seuchenartigen Pferdesterbens in Westpreussen. Cent. f. Bac. u. Par. I, XXIX, p. 285, 1901.

The authors investigate a somewhat unusual epidemic among horses occurring in West Prussia, and causing the death of quite a number. The disease

was accompanied by fever and intestinal troubles, and lasted from two hours to eight weeks in different cases. Post mortem examination showed the presence in the intestines, of ulcers which had a tendency to perforate the wall. Bacteriological study of the infected parts showed exceptionally large numbers of a

bacillus which the authors regard as the true *coli bacillus*. Finding this bacillus in such large numbers, the authors were led to experiment with it, and succeeded in demonstrating that cultures of the bacillus were pathogenic for the horse. By the use of these cultures, partly with food and partly by intravenous inoculation, they succeeded in reproducing the disease in experimental animals. They are of the conclusion, therefore, that the widespread *coli bacillus* is occasionally the cause of serious and fatal epidemics among horses.

H. W. C.

Bienstock. Du rôle des Bactéries de l'intestin.
Ann. de l'Inst. Past. XIV, p. 750, 1900.

The author gives a very suggestive paper upon the functions of the ordinary intestinal bacteria. He has previously shown in the intestine of animals the presence of a *Bacillus putrificus*, which produces a putrefying action on proteids. He now finds that, under normal conditions, such putrefaction of the contents of the intestine does not occur. This fact seems surprising, inasmuch as *B. putrificus* is constantly present in the intestine, and the conditions are apparently proper for its growth. Bienstock is of the opinion that putrefactive action is checked by the presence, in the intestine, of certain aerobic bacteria, such as *lactic* and the *coli* bacilli. Experiments show that the putrefaction produced by *B. putrificus* does not take place when a quantity of these aerobic bacteria are present. The author concludes, therefore, that these aerobic bacteria, which are uniformly found in the normal intestine, are of direct value to the human body in preventing the putrefaction of the intestinal contents. He points out the fact that sterilized, and even pasteurized, milk is not so readily digested as raw milk, especially by persons with intestinal disturbances, and this he attributes to the fact that since the heat has destroyed the lactic organisms, these organisms are not present in the intestine to prevent the *putrificus* from producing putrefaction. In short, the author concludes that the reason why ordinary micro-organisms are needed in the intestine is to prevent the putrefaction which would otherwise occur in the intestinal contents, owing to the presence of certain putrefying micro-organisms which are always found.

H. W. C.

Reed, Carroll and Agramonte. The Etiology
of Yellow Fever. Med. Rec., Feb. 16, 1901.

These authors have presented a further report upon their conclusions in regard to the relation of yellow fever to mosquitoes. The results reached are of immense importance and are too numerous to be summarized. The most important are, that the disease is transmitted from yellow fever patients to healthy persons by the bites of mosquitoes, there being a period of incubation from 41 hours to 5 days. They have repeatedly succeeded in reproducing the disease by allowing mosquitoes (*Culex fasciatus*) to bite patients and, subsequently, healthy individuals. They find that an attack of yellow fever conveyed by a mosquito bite confers immunity against disease. A house is only infested with yellow fever when containing no mosquitoes. Yellow fever is not distributed by soiled articles of clothing or bedding, as has been supposed. The spread of yellow fever may be most effectually prevented by protecting the patient from mosquito bites. These conclusions, which represent only a few of

the important results of the work of these investigators, are clearly of the utmost importance in the future study of this serious disease. H. W. C.

Hilsum. Bakteriologische Untersuchung eines Schwimmbades in Bezug auf Selbstreinigung. Cent. f. Bak. u. Par I, 2: 661, 1901.

The author studies the bacteria present in a swimming bath which was in constant use. He finds that the number of bacteria increased regularly during the first day, after being newly filled with water, and then constantly decreased. This decrease in the number of bacteria, he points out, could be due neither to the action of light nor to sedimentation, since the number of bacteria at night and morning was essentially the same, and since the water was in constant use, a condition which would prevent sedimentation. Nor does he believe that a want of food can be the cause, since the water filtered through a pasteur filter is an excellent culture medium for bacteria. The author believes that the matter is one of struggle among different bacteria with each other, resulting in a destruction of many individuals. H. W. C.

NOTES ON RECENT MINERALOGICAL LITERATURE.

ALFRED J. MOSES AND LEA MCI. LUQUER.

Books and reprints for review should be sent to Alfred J. Moses, Columbia University, New York, N. Y.

Viola, C. Ueber das "Glaukisiren" verschiedener Feldspäthe. Zeit. f. Kryst. 34: 171-195, 1901.

The writer proposes the term "Glaukisiren" for the variety of schillerization which takes place in moonstone

—that is, when the inner reflection produces a silvery or bluish light. Whether or no a convenient English form of this term will be made remains to be seen, but Glaukisiren may be translated as the process which produces the silvery schiller or inner reflection. Hitherto the assumption has been that the process was one of internal reflection and interference. The tests made by Viola tend to prove that instead of interference it is a process of absorption. The method followed in examining the moonstone of Ceylon was as follows:

Ceylon moonstone consists of coarse feldspar crystals, enclosing and intergrown with quartz. The feldspar is not absolutely definite, but the analyses indicate orthoclase with slight admixture of lime and soda. It is usually milk white in color, and the cleavages are wave-like. The silvery schiller appears to be most marked parallel to the face $\bar{2}01$. Sections were therefore cut parallel to this face, and about 1 mm. thick. These were mounted in an ordinary Fuess goniometer with $\bar{2}01$ vertical, and the plane of reflection of the schiller (found by experiment to be approximately (010)) horizontal. Parallel light from the collimator, reflected from $\bar{2}01$ as parallel light, gave a sharp signal for the plane; the section was then revolved until the sky-blue schiller signal was obtained; this was not sharp, but diffused through four to five degrees, but the

brighter center could be determined within one degree.

It was found that the signal was obtained for all angles of incidence precisely as if it was due to a reflection from some interior surface, and it might be assumed that the diffusion was due to this surface not being perfectly smooth. In order to find the angle between the supposed interior surface of reflection and the surface of the section,

Let O be center of revolution,

Let C be collimator,

Let T be telescope,

Let N be normal to plate when yielding ordinary signal,

Let N' be normal to plate when yielding schiller signal.

If $COT = 2\varphi$ then $CON = TON = \varphi$ and, see Fig., $a' = \varphi - e$ and $b' = \varphi + e$. But as the rays from C and to T must have been refracted, the true incident and reflected rays for the internal surface cannot have been these, but rather such rays as $C'O$ and $T'O$, making angles a and b with the surface normal ON' , in which $\sin a = \frac{\sin a'}{n}$ and $\sin b = \frac{\sin b'}{n}$, n being the mean index of refraction for the moonstone. The normal to the internal surface will therefore be ON'' , and the angle between the two surfaces will be equal to $N'O N''$, denoted by d , and, from the figure, $b - d = a + d$, or $d = \frac{b - a}{2}$.

For Ceylon moonstone cut parallel $\overline{201}$ $d = 12^\circ 5'$ and $65^\circ 19'$, and is essentially independent of the angle of incidence. There is also a *transmitted* image the color of which is complementary, that is, yellowish to reddish orange. This would indicate that the violet and blue rays were diffused and reflected, while the red, yellow, etc., penetrate. If the phenomenon were one of interference, then if for a certain angle of incidence the reflected color is blue, it must pass into red for a larger incident angle, and this it does not do. The theory of internal reflection and absorption seems to best explain the phenomena. Similar results were obtained with the Amelia Co., Va., albite and the adular of Zillertal.

A. J. M.

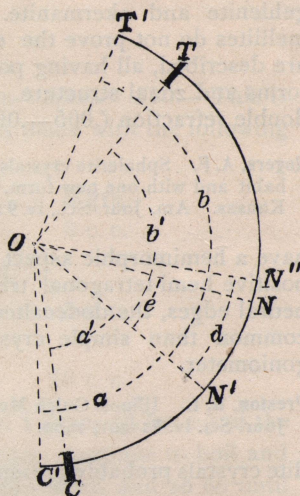
Melonite. From Worturpa, South Australia.
Trans. Roy. Soc. S. Australia, **23**: 211, 1899.

Thin lamellæ with bright metallic lustre. Color on cleavage, silver white with normal incidence, reddish brown with oblique incidence. G. 7.6 H. 1.5. Streak lead gray. $Ni_2 Te_3$ with traces Pb, Bi, and Au.

Minerals of Japan. Kotora Jumbo, in Jour. Coll. Sci. Tokio, **11**: 213-281, 1899. Nine page Abs. Zeit. f. Kryst. **34**: 215, 1901.

Fouqué, F. Contribution à l'étude des minéraux de group de la mélilite. Bull. Soc. Min. **23**: 10, 1900.

According to Vogt there should exist a series of minerals, belonging to this group, low in silica and containing variable amounts of calcium and aluminium, varying between the limits of



gehlenite and akermanite. Author's investigations on artificially prepared melilites do not prove the existence of this series. Different artificial melilites are described, all having positive optical character, and some showing spherulitic forms and zonal structure. They are also characterized by strong (for melilite) double refraction (.005—.006).

L. McI. L.

Rogers, A. F. Sphalerite crystals of a peculiar habit and with one new form, from Galena, Kansas. *Am. Jour. Sci.*, iv. 9: 134, 1900.

The crystals are reddish-brown in color, and shortened in the direction of one of the octahedral interaxes. They

have a hemimorphic aspect due to the presence of the faces of the new form, a positive hemi-tetragonal trisoctahedron σ (833), truncating half of the dodecahedral edges, the dodecahedron d (110) being the chief form. Twins are more common than simple crystals. Measurements were made only with contact goniometer.

L. McI. L.

Preston, H. L. Illinois Gulch Meteorite. *Am. Jour. Sci.* iv. 9: 201, 1900.

Troilite present in very small quantity. Iron shows no etching figures. Rhombic crystals probably present. Weight = 2.435 grams. $G=7.7$. Analysis given.

L. McI. L.

Sulvanite, a new mineral. G. A. Goyder. *Jour. Chem. Soc.*, 77: 1094, 1900.

A copper "sulpho-vanadite," $3\text{Cu}_2\text{S} \cdot \text{V}_2\text{S}_5$, from "near the Burra, in South

Australia," associated with malachite, azurite, quartz, vanadium ochre, gypsum, and calcite. First recorded instance of a sulphide mineral containing vanadium as one of its principal constituents. Two analyses gave the following:

	Cu	V.	S.
A, - - - - -	51.57	13.46	34.97
B, - - - - -	52.96	13.72	34.62
Theoretical for $3\text{Cu}_2\text{S} \cdot \text{V}_2\text{S}_5$, -	51.50	13.88	34.62

Some of the physical properties are: massive, luster metallic to sub-metallic, color bronze yellow, streak nearly black. $H=3.5$ $G=4$. No crystalline form detected.

A. F. R.

Richards, Joseph W., and Powell, Norman S. Substitutes for Hydrochloric Acid in Testing Carbonates. *Jour. Am. Chem. Soc.* 22: 117, March, 1900.

The experiments were undertaken to find a satisfactory substitute for hydrochloric acid for testing carbonates in

the field. The action of 20 per cent. solutions of potassium acid sulphate, citric acid, tartaric acid, and 10 per cent. solution of oxalic acid, on sixteen of the more common carbonate minerals, in lump and powder, are recorded in tabular form. Tartaric acid is regarded as the best reagent, with citric acid as a close second. Some sulphides give off hydrogen sulphide with the reagents. The authors seem to have been utterly unaware of Bolton's previous work along this line. *Annals N. Y. Acad. Sci.*, 1: 1, 1879; *Chemical News*, 36: 249; 37: 14, 24, 65, 86, 98; 43: 31, 39.

A. F. R.

Nichols, Henry W. A New Test for Chlorine for Use with the Blowpipe. *Amer. Chem. Jour.* 25: 315, April, 1901.

To test a substance for chlorine it is powdered with potassium acid sulphate and placed in a closed tube. A frag-

ment of filter paper, moistened with cobalt nitrate solution, is put into the mouth of the tube, and the mixture fused. If chlorides are present the paper will turn a bright blue, and if bromides or iodides are present the color will be green. In the case of minute quantities it may be necessary to dry the paper before the color appears. Other details of manipulation are given. Specimens of sodalite (Cl. 7.3 per cent.), zunyite (Cl. 2.9 per cent.), and apatite gave good reactions.

A. F. R.

MEDICAL NOTES.

METHODS FOR THE DETECTION OF SUGAR IN URINE—*Haine's Test*.—This is considered the best of the copper tests for sugar, and is made with the following solution :

Copper sulphate,	-	-	-	-	-	6 grs.
Water, distilled,	-	-	-	-	-	3 c. c.

Dissolve the CuSO_4 thoroughly in the water and add,

Glycerin, pure,	-	-	-	-	-	3 c. c.
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which should be thoroughly mixed, after which add,

Liquor potassæ,	-	-	-	-	-	30 c. c.
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To make a test for sugar in a sample of urine, boil 1 dram (3.7 c. c.) of the solution in a test tube, and add 2 or 3 drops of the urine; continue to boil and if, after a few seconds, no reaction occur, add 2 or 3 drops more, and so on until 8 drops are added, after which no more urine should be added. It is best to use the least possible amount of urine that will produce the reaction. The solution should not be allowed to boil more than one-half minute. If sugar is present in the urine, a yellow or yellowish-red precipitate forms.

This test is simple in application, and is sufficiently reliable to be depended upon in general practice. The solution is perfectly stable, and may be kept indefinitely without deteriorating.

Phenyl-Hydrazin Test.—This is an exceedingly delicate test, and is very desirable when the routine test, above, leaves any doubt as to the presence of sugar in the urine. The test is performed by adding to 50 c. c. of the suspected urine, 2 gms. of phenyl-hydrazin hydrochloride, 1.5 gm. of sodium acetate, and 20 c. c. of distilled water. This solution should be heated moderately in a water bath for an hour, after which, when cooled, if the smallest amount of sugar be present, a yellowish crystalline precipitate is deposited.

With the above methods the presence or absence of sugar in the urine may be readily and conclusively ascertained. When sugar is detected, it is very important to determine the amount present. This may be accomplished with accuracy by pursuing the following method :

Purdy's Method for the quantitative determination of sugar in urine.—Dissolve, with gentle heat, .5 gm. pure cupric sulphate, and 3.8 c. c. glycerol in 20 c. c. distilled water. With this mix a solution of 2.4 gms. potassium hydroxide in 20 c. c. distilled water, and add 35 c. c. strong ammonia. Make up to 100 c. c. with distilled water.

Place exactly 35 c. c. of this solution in a flask, dilute with an equal amount of distilled water, and bring to boil. To this add *slowly*, drop by drop, the urine to be tested until the solution loses its blue color and becomes perfectly colorless. The amount of urine required contains exactly .02 gm. of sugar. If it takes 1 c. c. of urine, there is 2 per cent. of sugar; if it takes $\frac{1}{4}$ c. c. of urine, there is 8 per cent. of sugar.

C. W. J.

NEWS AND NOTES.

The article "The University of Montana Biological Station," which appeared in the May number of the JOURNAL, elicited a number of questions, in answer to which the author, Prof. Morton J. Elrod, has added the following:

The microscopical equipment during the past summer consisted of four large compound microscopes, with two-thirds and one-sixth objectives each; one small microscope with three objectives; several additional objectives of higher and lower powers; a dozen or more hand lenses, doublets; an abundance of glass slips and covers; an assortment of common stains and chemicals; glassware necessary to carry on the work, such as watch glasses, small beakers, pipettes, staining dishes, etc. A centrifugal apparatus was used to determine the quantity of the plankton. The camera had a Zeiss anastigmat lens, Series IV, telephoto attachment, and ray filter. The vials for containing microscopic life, plankton, were of three different lengths, with the same diameter, making it necessary to carry corks of one size only. This was found to be a great convenience, especially as several gross of vials were carried. The vials were straight shells, without neck. As a preservative formaldehyde was used. The concentrated or forty per cent. solution was carried in small bottles, so that if one should be broken but a portion of the supply would be lost. The concentrated solution was diluted as used. No alcohol could be carried while collecting, the laws preventing alcohol from being taken into an Indian reservation.

During the past summer seventeen students took advantage of the facilities for work offered by the station. The microscopical work was largely elementary. Much use of the instrument was made in the study of Entomostraca from the lakes. Animal and plant structures were examined, several students using the microscope for the first time. Simple mounts were made, and the method of using stains was made known through practice. Four of those attending took regular courses in either botany or general zoölogy, with daily use of microscope and microscopic material. One microscope was in constant use for two months in study of Entomostraca. Four students devoted most of the summer to ornithology. One worked on fishes, one on butterflies.

In the light of past experience the following conclusions have been reached. Although the state is large and the population small, there is much more interest shown in the station work than was at first anticipated. The obstacles in the way are not so great as would naturally be expected. The chief difficulty is in getting over the country, but if one is not crowded for time this makes little difference. The lakes in the mountains, though containing cold water, have many very interesting forms of life. Very few of the lakes have been touched. Flat-head lake, with its inlets and outlet, has sufficient territory for a large working force, and sufficient material for wide range of study and experiment. Naturalists from the East who have a month to spare in the summer and who want to see the West, and at the same time wish to do some work, may find the station of advantage, and will be able to get into the field and into the hills without wasting most of the time in learning how and where to go. The field is rich enough to

warrant greater expenditure in apparatus and material. A house boat would be a great convenience and of great utility. The great needs of the station, in order to secure the best results, are more extensive working material and a longer working period. For many years the station will be a place for investigation rather than a summer school for students. Its best work will be done by making provision for both. There is excellent opportunity to establish a station on a larger scale, in a region offering great variety of life, from Alpine to that at 3000 feet altitude, and from swamp to barren hill. It is hoped the income of the university will warrant the increased expenditure at an early date. While there is no comparison between the life of the region and that of a favorable ocean locality, the problems offered are of a different nature, fully as interesting, and quite as important. Nowhere is there better opportunity to study variation and its effects than in mountain regions.

The work of the coming season will be better than in preceding years. In addition to the work of the director, Principal P. M. Silloway, of the Fergus county, Montana, Free High School, will have charge of work in ornithology, which he did so ably the past season. Maurice Ricker, principal of the Burlington, Iowa, High School, will give the instruction in nature study and physiography. The New York Botanical Garden will coöperate in the botanical work of the station. Dr. D. T. MacDougal, director of the laboratories in that institution, will join the party in the field for the purpose of making collections and pursuing some investigations upon the results of climate and vegetation, and will continue both lines of work at the station. The botanical work during the session will be under his guidance. Attention will be given to general botany, and to the special features of the flora of Montana. Mr. R. S. Williams, of the same institution, will spend the month of June making collections in the northwestern part of the state, and will be present during a part of the session, giving special attention to mosses and ferns.

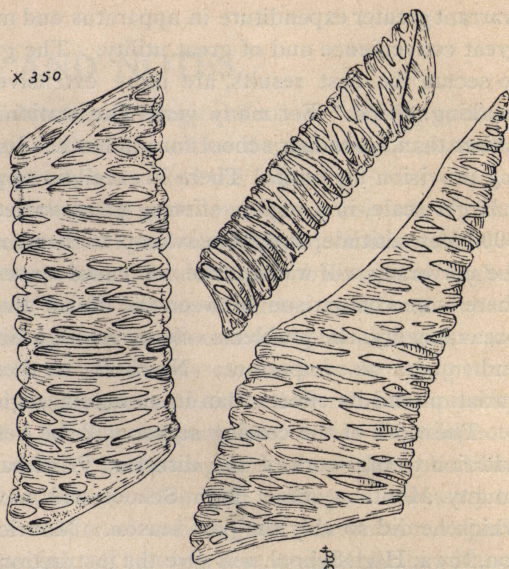
During the five or six weeks previous to the opening of the station the instructors will devote their time to collecting in the immediate region. An outfit has been provided which will make the trip quite comfortable, and the entire time will be devoted to collecting in different fields, from snowy mountain summits to the marshes of the lakes and rivers. During this trip additional collections will be made in lakes not yet visited, and in regions where collectors have not yet been. After the collecting trip the party will proceed to the station, and will take care of the students attending, at the same time continuing the collecting and making additional observations.

The collecting trip is made possible through a contribution from Hon. Wm. A. Clark, who has contributed annually for this purpose, and to whom the station is greatly indebted. The purchase of the boats, erection of building, and expense of the instructors during the past two years has been met by contributions from friends, the principal contributors being E. L. Bonner, H. W. Hammond, A. B. Hammond, Dr. W. P. Mills, W. P. Murphy, and W. A. Clark. The expense for the coming summer, in addition to the contribution by Senator Clark, will be met by university appropriation.

DEMONSTRATION OF RETICULATE VESSELS.—The teacher of plant histology is usually seeking for the best possible materials to illustrate the several kinds of cells that are to be examined by his students. For well defined reticulate vessels, I have seen nothing to equal those found in the thickened roots of *Arenaria striata*, which grew in our botanic garden. They are somewhat different from those found in the stems of *Impatiens*.

W. J. BEAL.

x 350



In the cuts of starchy granules of the pea, they are represented as having cracks, or checks in the middle. I wonder if it is generally known that the checks seldom appear if the peas are placed in alcohol or glycerin before drying?

W. J. BEAL.

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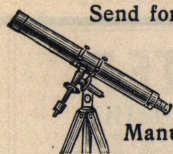
5. Will you kindly refer me to a good method of how to make a biologic aquarium? In the JOURNAL OF APPLIED MICROSCOPY are a few notes on Cultivation of Algæ, but not sufficient for an amateur. What should be the soil—gravel, sand, or mud from a pond? How proceed to stock it and with what? Is the evaporation to be supplied from a pond or hydrant? If glass cover on, can there be enough air to pass between for living? I am desirous of having a number of jars for general elementary biology work; to supply *Amœba*, *Hydra*, *Chara*, *Vorticella*, *Spirogyra*, *Vaucheria*, *Nitella*, *Vallisneria*, etc.—V. A. L.

6. What is Scott's method for the examination of blood?—T. G. S.

7. Can tinted paper be used for the hæmoglobin test?—T. G. S.

REPLY TO QUESTIONS 3 AND 4 IN THE MAY NUMBER.

The Welsbach light is practically worthless for high-power microscopy, either visual or photographic, for with condenser focused, as it should be, an image of the fabric of the mantle is projected into field of view. Have found no difficulty in making photographs up to $\times 1200$, using H. I. objectives and Huyghenian oculars, with oil lamp, half-inch wick. Render rays approximately parallel with bulls-eye, or better, a large size, short focus photographic lens placed at its focal distance from lamp flame, and converge on object with substage condenser sharply focused, achromatic condenser decidedly preferable, but Abbe condenser will answer fairly if nothing better is available. No noticeable advantage derived from the use of achromatic, periscopic, orthoscopic, or compensation oculars over the Huyghenian, when used with achromatic objectives.—F. J. K.



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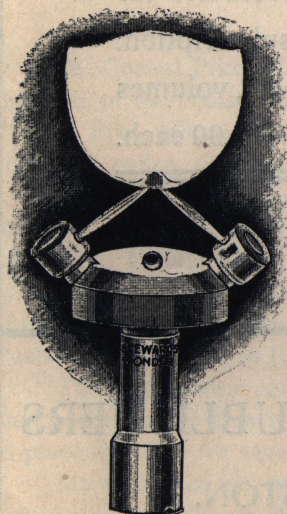
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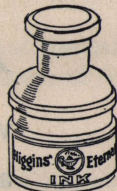
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Original Articles in the December Number, 1900.

ITO, T.—*Plantæ Sinenses Yoshianæ*, X.

ASO, K.—A Physiological Function of Oxydase in Kaki-Fruit.

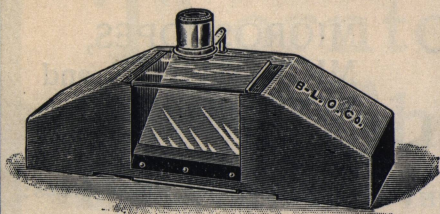
ICHIMURA, T.—*Pflanzenverbreitung auf dem Tateyama in der prouing Etchu.*

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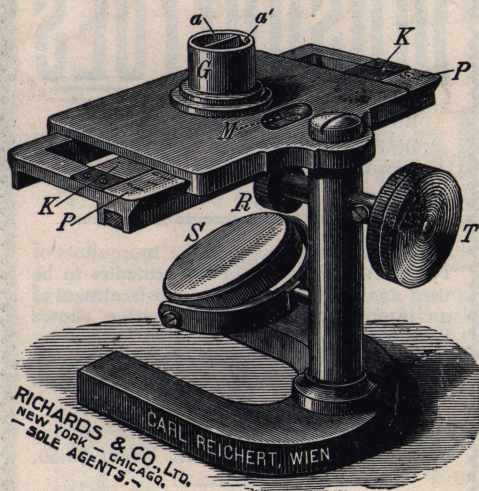
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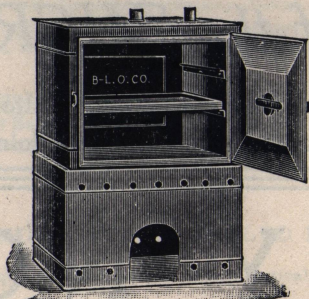
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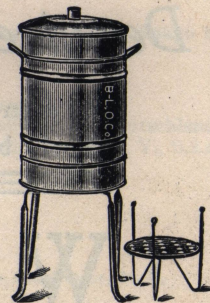
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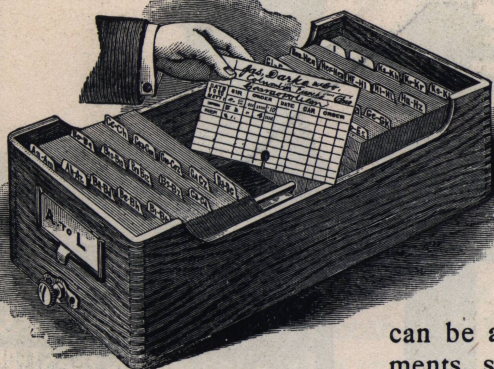
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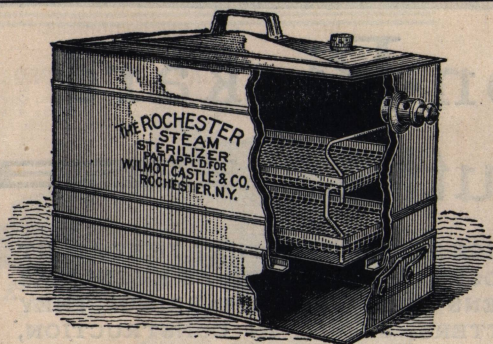
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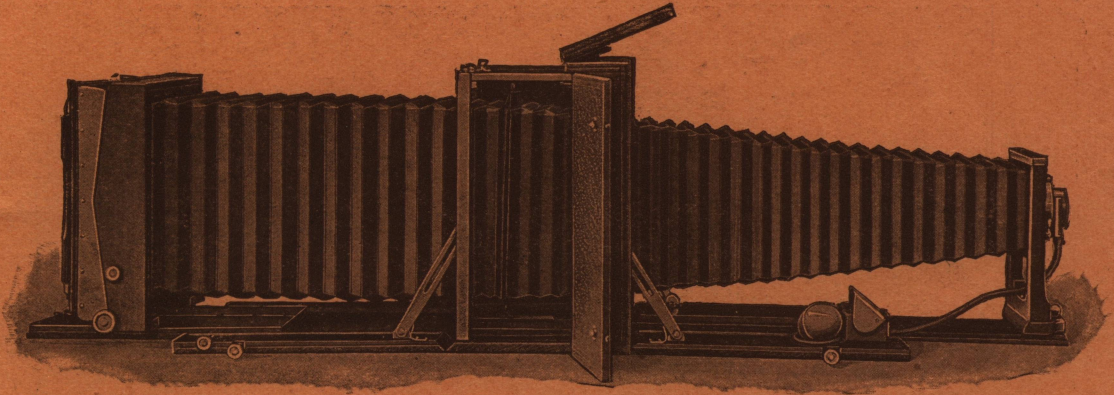
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